ETIOLOGY OF GASTROENTERITIS IN TWO POPULATIONS IN PERU: MEDICALLY-ATTENDED YOUNG CHILDREN AND A PERUVIAN MILITARY COHORT

by
Sarah-Blythe Ballard, MD, MPH

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PREFACE

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ABSTRACT

Globally, norovirus is the most common cause of acute sporadic gastroenteritis in adults and the second leading cause of severe diarrhea in children, after rotavirus. Vaccine development against norovirus is currently underway. Successful vaccination strategies against norovirus will require understanding the burden of disease and relevant genotypes in target populations. However, due to the cost associated with molecular diagnostics, few data are available from populations living in low- and middle-income countries (LMIC). We conducted epidemiological studies in two populations. (1) To evaluate the burden of disease, predominate genotypes, and associated symptoms among adults living in an LMIC setting, we conducted a nested case-control study within a Peruvian military cohort from 2004 through 2011. We then used the epidemiological data from this study to evaluate the cost-effectiveness of a potential vaccine against norovirus in this adult population. (2) We then conducted a case-control study in the national children's hospital in Peru following the universal implementation of a vaccine against rotavirus in order to determine the a) pathogen-specific attributable risk among children with medically-attended gastroenteritis; b) relationship between pediatric infection with specific pathogens and gastroenteritis severity; and c) genetic diversity of circulating norovirus, sapovirus, and rotavirus. We found that, after *Shigella,* norovirus was the leading cause of diarrhea in the adult Peruvian military population, contributing to a disease attributable fraction (AF) of 6.4%. Relative to implementation in the United States, we calculated *Shigella* vaccine implementation to be significantly more cost-effective for the Peruvian military (CER_{DDL}=$350

versus $1275), with norovirus vaccine implementation being moderately more cost effective (CER_{DDL}=$926 versus $1344). In the pediatric hospital population, we found that the following six pathogens were independently positively associated with gastroenteritis: norovirus GII (AF 29.1, 95% CI: 28.0-32.3); rotavirus (AF 8.9, 95% CI: 6.8-9.7); sapovirus (AF 6.3, 95% CI: 4.3-7.4); ETEC St=/Lt+St+ (AF 2.4, 95% CI: 0.6-3.1); *Shigella* (AF 2.0, 95% CI: 0.4-2.2); and astrovirus (AF 2.8, 95% CI: 0.0-4.0). Caliciviruses are an important cause of diarrhea among children with medically-attended diarrhea in Peru, as well as adults in special populations, such as the military. Control strategies should consider multivalent vaccines that target these pathogens.

TABLE OF CONTENTS

I. REVIEW: Current concepts in tropical and travel-associated norovirus gastroenteritis……1

II. FIRST PAPER: Epidemiology and genetic characterization of noroviruses among adult Peruvian military personnel in an endemic setting, Amazon Basin, 2004-2011……18

III. SECOND PAPER: Application of a relative cost-effectiveness analysis model to the evaluation of pathogen-specific vaccines against *Campylobacter*, ETEC, *Shigella*, and norovirus to a Peruvian adult military population in the Amazon Basin……33

IV. THIRD PAPER: Etiology of medically-attended gastroenteritits in children younger than five years of age following universal rotavirus vaccine implementation in Peru……41

V. CONCLUSIONS……60

VI. TABLES:

Table 1. Pathogens Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin……63

Table 2. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Peruvian Military Personnel in the Amazon Basin……64

Table 3. Norovirus Genotypes/Variants Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin……65

Table 4. Pathogens Detected in Peruvian Military Personnel in the Amazon Basin with Norovirus-positive versus Norovirus-negative Stool Samples……66

Table 5. Clinical Features of GI- versus GII-associated Norovirus Gastroenteritis in Peruvian Military Personnel in the Amazon Basin……67

Table 6. Parameter Estimates Used for the Pathogen-Specific Gastroenteritis Vaccine Cost Effectiveness Analysis Applied to the Peruvian Military Population….68

Table 7. Baseline Estimates of Total Costs, Effectiveness Measures, and Cost-Effectiveness Ratios for Pathogen-Specific Analysis of Vaccines Against *Campylobacter,* ETEC, *Shigella,* and Norovirus in a Peruvian Military Population in the Amazon Basin……69

Table 8. Population Characteristics and Pathogens Detected in Stool Samples from Children Younger than Five Years of Age with and without Medically-attended Gastroenteritis in Peru……70

Table 9. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Children in Lima, Peru……72

Table 10. Clinical Features and Severity of Gastroenteritis by Pathogen in Children Younger than Five Years of Age in Peru……73

VII. FIGURES:

Figure 1. Map of Peru, Indicating the Peruvian Military Study Site of Iquitos in the Peruvian Amazon Basin and Lima, the Capital City where the National Children's Hospital is Located……75

Figure 2. Flow Diagram of Peruvian Military Participant Enrollment and Selection of Participants for the Nested Case-Control Study……76

Figure 3. Economic model evaluating the cost-effectiveness of vaccines against specific gastroenteritis-associated pathogens for a Peruvian military population, taken from (Tallant et al. 2014a)

Figure 4. One-way Sensitivity Analyses for the Cost-Effectiveness Analyses of Vaccines Against (A) *Campylobacter*; (B) ETEC; (C) *Shigella*; and (D) Norovirus in the Peruvian Military Population in the Amazon Basin……78

Figure 5. Rotavirus prevalence over study period demonstrating the relationship between rotavirus seasonality (red line) and rotavirus genotype G12P[8] prevalence (green line), Peru, 2013-15……80

VIII. REFERENCES……81

IX. *Curriculum vitae*……117

I. REVIEW: CURRENT CONCEPTS IN TROPICAL AND TRAVEL-ASSOCIATED NOROVIRUS GASTROENTERITIS

INTRODUCTION

Globally, norovirus is the most common cause of acute sporadic gastroenteritis in adults and the second leading cause of severe diarrhea in children, after rotavirus. In countries with mature rotavirus immunization programs, norovirus is emerging as the predominant diarrhea-associated pathogen in young children (Pringle et al. 2015). This section covers the latest scientific insights into norovirus infections in the tropics while addressing the challenges of controlling this disease in endemic populations and travelers to these regions.

ASSESSING NOROVIRUS DISEASE BURDEN IN THE TROPICS

Norovirus detection in symptomatic cases. Ahmed and colleagues estimated a global norovirus prevalence of 18% among acute gastroenteritis cases in a meta-analysis of 175 studies published between 2008 and 2014 (S. M. Ahmed et al. 2014). The pooled prevalences among hospitalized and community cases were 17% and 24%, respectively (S. M. Ahmed et al. 2014). When stratified by World Health Organization (WHO) mortality category (Beaglehold and Prentice 2003), norovirus was more prevalent in diarrhea stools from low-mortality than high-mortality developing settings (19% versus 14%, respectively) (S. M. Ahmed et al. 2014). This likely represents a more diverse enteropathogen landscape in the context of higher overall diarrhea incidence in high-mortality settings (S. M. Ahmed et al. 2014). Prior to rotavirus vaccine implementation, norovirus was the most frequently identified pathogen in ambulatory (Estévez et al. 2013) and community (Saito et al. 2014)

diarrhea cases in certain low- and middle-income country (LMIC) settings. Rotavirus was usually reported more frequently in hospitalized children (Estévez et al. 2013), although up to 55% of hospital diarrhea cases demonstrated human calicivirus (norovirus and/or sapovirus) infection when evaluated with both immunologic and molecular detection methods (Parashar et al. 2004; Cama et al. 1999). Following successful universal rotavirus vaccination in LMICs, norovirus is recognized as the predominant pathogen in hospitalized (Bucardo et al. 2014), outpatient (Estévez et al. 2013), and community (Becker-Dreps et al. 2014) diarrhea cases. The relationship between norovirus infection and adult diarrhea has not been previously evaluated LMIC military service members (Ballard, Reaves, et al. 2015a).

Norovirus detection in asymptomatic individuals. The detection of norovirus in stools from asymptomatic individuals complicates disease burden estimates. Globally, the pooled asymptomatic prevalence from the 20 controlled studies in Ahmed's meta-analysis was 7% (S. M. Ahmed et al. 2014). Fifteen to 35% of norovirus infections are asymptomatic, but both symptomatically- and asymptomatically-infected individuals shed virus at similar levels for similar amounts of time, although duration may vary by genotype and variant (Saito et al. 2014). Host genetic factors, such as the absence of the alpha-1,2-fucosyltransferase (FUT2) enzyme in "secretor negative" individuals, appear to confer absolute protection to infection to specific variants (Ben A. Lopman et al. 2015). Other host factors, such as histo-blood group antigen polymorphisms, result in heterogeneous

susceptibility to norovirus infection (Ben A. Lopman et al. 2015). Following infection, viral shedding lasts approximately 20-30 days in adults (M. M. Levine and Robins-Browne 2012). Excretion can be prolonged in children, the elderly, and immunocompromised, who serve as reservoirs for transmission (Sukhrie et al. 2010) and may also contribute to the emergence of novel epidemic variants (Pringle et al. 2015). In Saito and colleagues' Peruvian birth cohort, norovirus excretion was longer for genogroup (G) II (median 34.5 days; maximum 98) than GI (median 8.5 days; maximum 49) (Saito et al. 2014). Both symptomatic and asymptomatic infections during the first year of life were associated with linear growth deficits that persisted into the second year of life (Saito et al. 2014).

In the absence of longitudinal data, distinguishing symptomatic from asymptomatic norovirus infections is difficult. Asymptomatic individuals tend to have higher RT-PCR cycle thresholds values than individuals with acute gastroenteritis, but there is no clear viral load cutoff that corresponds with symptom resolution (Phillips et al. 2009). To illustrate the marked increase in asymptomatic norovirus prevalence resulting in small increases in basic reproduction number, Lopman and colleagues created a dynamic norovirus transmission model of norovirus infection, immunity, and disease (B. Lopman et al. 2014). In this model, the case: control prevalence ratio was high in developed settings and decreased dramatically in a high-exposure scenario with the same disease incidence (B. Lopman et al. 2014). This could explain why the Global Enteric Multi-Center Study (GEMS), a case-control analysis of diarrhea in the tropics, noted similar frequencies of norovirus in case and control

stools, ultimately dermined that norovirus contributed minimally to moderate-to-severe diarrheal disease (Kotloff et al. 2013). In contrast, longitudinal studies that more clearly distinguish symptomatic and asymptomatic infections demonstrate higher burdens of norovirus-associated diarrhea in similar developing settings (Saito et al. 2014; B. Lopman and Kang 2014). In their Peruvian birth cohort, Saito and colleagues calculated a norovirus attributable diarrheal disease fraction of 7.8% in the first and 23.1% in the second year of life (Saito et al. 2014).

Defining norovirus disease and severity. The lack of standard norovirus case definitions and clinical severity measures complicate disease burden estimation and comparative intervention evaluations in tropical settings. Historically dubbed "winter vomiting disease," norovirus often causes emesis in the absence of diarrhea. As a result, diarrhea-based gastroenteritis case definitions likely underestimate disease burden by excluding vomiting-only disease. Of the 175 studies included in Ahmed and colleagues' meta-analysis of norovirus gastroenteritis, 143 (82%) either did not provide a case definition for acute gastroenteritis or excluded vomiting-only disease. Clinical severity measures are most commonly based on the 20-point Vesikari scale, which dichotomizes gastroenteritis into "mild" (<11) or "severe" disease (Vesikari, Giaquinto, and Huppertz 2006; Ruuska and Vesikari 1990). Other measures of disease severity include the 24-point Clark scale (H. F. Clark et al. 1988), modified Vesikari scales (Saito et al. 2014), the World Health Organization scale ("The Treatment of Diarrhea. A Manual for Physicians and Other Senior Health Workers. Fourth Revision, 2005" 2005), and severity scores based on signs and

symptoms (Monica et al. 2007; El Qazoui et al. 2014; Abugalia et al. 2011; Chhabra et al. 2009; Farkas et al. 2000; Gutiérrez-Escolano et al. 2010), reported symptoms (Yang et al. 2010; Räsänen et al. 2011; Sdiri-Loulizi et al. 2008; Flores et al. 2011)(28 Yang, 29 Rasanen, 30 Sdiri-Loulizi, 31 Flores), length of hospitalization (Räsänen et al. 2011; So et al. 2013) and impact on daily activities (Lalani et al. 2015). Different gastroenteritis case and severity definitions can bias results against specific pathogens. For example, defining "moderate-to-severe" diarrhea as the presence of sunken eyes, loss of skin turgor, intravenous fluid prescription, dysentery, or hospital admission, GEMS reported that norovirus contributed minimally to moderate-to-severe disease (Kotloff et al. 2013). In contrast, O'Ryan and colleagues' hospital-based study of diarrheal disease in Chile reported that norovirus was a leading cause of moderate-to-severe disease, as defined by a Vesikari score greater than 6 (of 20 possible points) (Ruuska and Vesikari 1990; O'Ryan et al. 2010). Using uniform case definitions and severity measures for norovirus vaccine efficacy studies will allow direct comparison of results, avoiding the possibility that different results might be attributable to the use of differing scales, as was the case when Rotateq® and Rotarix® were evaluated using the Clark and Vesikari scales, respectively (Givon-Lavi, Greenberg, and Dagan 2008).

Malnutrition and norovirus infection. Undernutrition affects one in five children in the tropics and has been associated with half of deaths in children younger than five years worldwide (Bryce et al. 2017). Poor nutrition weakens host immune responses and alters the gut microbiota, both of which can worsen the clinical

course of diarrheal disease (Hickman et al. 2014). After infecting well-nourished and protein energy deficient mice with murine norovirus, Hickman and colleagues found that malnourished mice demonstrated more weight loss, reduced antibody responses, loss of protective immunity, and enhanced viral evolution (Hickman et al. 2014). Although the well-nourished mice fared better in terms of disease severity, norovirus infection resulted in a gut microbial environment similar to that of malnourished mice (Hickman et al. 2014). Human studies are currently being conducted by the Interactions of Malnutrition & Enteric Infections: Consequences for Child Health and Development (MAL-ED) group, but results are pending (MAL-ED Network Investigators et al. 2014; Richard et al. 2014; Houpt et al. 2014; J. A. Platts-Mills et al. 2014; T. Ahmed et al. 2014; Lima et al. 2014; John et al. 2014; Shrestha et al. 2014; Turab et al. 2014; P. P. Yori et al. 2014; Bessong et al. 2014; Mduma et al. 2014). Particularly relevant to assessing tropical norovirus infections are investigations that assess the role of the gut microbiota in nutritional status (Lang 2015), immune markers and vaccine failure in undernourished populations (Hoest et al. 2014; Keusch et al. 2014), tropical enteropathy and gut function (Hoest et al. 2014; Kosek et al. 2014; Denno et al. 2014), and the long-term impact of these factors on child development (MAL-ED Network Investigators et al. 2014; Murray-Kolb et al. 2014).

Norovirus coinfections. Individuals living in tropical settings often have intense exposure to enteric pathogens, and detection of more than one pathogen, particularly helminths, in stool samples is common. Helminth coinfections impair

viral immunity via an innate immunomodulation pathway. In this pathway, helminth-activated T_H2 cells release interleukin (IL)-4 and IL-13, which ligate IL-4 receptors on M2 macrophages (Osborne et al. 2014; Maizels and Gause 2014). This inhibits the production of virus-specific T-cells and greatly increases viral replication in macrophages (Maizels and Gause 2014). Helminth infections are also associated with changes in the gut microbiota (Walk et al. 2010; Rausch et al. 2013), although the extent to which these changes affect host responses to viruses and other enteropathogens is not clear. The type 2 immune response stimulated by helminth infection is also associated with enhanced tissue repair and reduced inflammation (Gause, Wynn, and Allen 2013), which could mitigate the severity of disease in coinfected individuals.

The molecular and cellular interactions between enteropathogens are important to consider when evaluating norovirus disease burden and pathogen-specific interventions (Kjetland et al. 2014). However, currently used qualitative pathogen detection (J. Liu et al. 2013; James A. Platts-Mills et al. 2014) and regression-based coinfection adjustment (Kotloff et al. 2013) methods are imperfect in the setting of asymptomatic infection, post-infectious shedding (Rao et al. 2001), and varying durations of immunity (Simmons et al. 2013). Probabilistic analytical methods that give weight to first infections and account for pathogen prevalance prior to symptom onset may present a clearer picture of pathogen-specific disease burdens in tropical settings where polymicrobial infections are detected frequently (Taniuchi et al. 2013).

NOROVIRUS IN TRAVELERS TO THE TROPICS

Up to 100 million people from high-income countries travel to the tropics each year, and 40-60% of them develop diarrhea (Harvey et al. 2013; de la Cabada Bauche and Dupont 2011). Norovirus is the second leading cause of travelers' diarrhea after Enteropathogenic *Escherichia coli* (ETEC) (Youmans et al. 2015). A systematic review of 51 published studies of travelers' diarrhea reported a pooled norovirus case stool prevalence of 6.6%, with higher norovirus prevalence in stools from travelers to Latin America (16.9%) and Africa (12.8%) than travelers to Asia (3.2%) (Beckmann et al. 2014). Norovirus has been associated with diarrhea in both children and adults returning from tropical settings (Beckmann et al. 2014), and norovirus coinfections with other pathogens, particularly ETEC, are common, (Gonzaga et al. 2011; Paschke et al. 2011). Travelers' diarrhea is associated with alterations in the gut microbiota that appear similar, regardless of the infecting enteropathogen (Youmans et al. 2015).

Norovirus afloat. Norovirus has a very low infectious dose (Teunis et al. 2008; Thebault et al. 2013; Kirby, Teunis, and Moe 2015; Atmar et al. 2014) and is extremely hard to eliminate from the environment (Parashar et al. 2004), making contamination of ships common. The US Centers for Disease Control and Prevention's Vessel Sanitation Program assists to prevent outbreaks on ships with foreign itineraries, where the impact of norovirus has been well documented (Beaumier 2007). During the 1990s, the implementation of sanitation measures

resulted in the reduction of norovirus outbreaks from 6.3 to 3.7 per 1,000 ship-weeks (Cramer, Gu, and Durbin 2003). In US military populations afloat, gastroenteritis outbreaks have been reported at nearly ten times the rate of vacation cruise ships, with an overall incidence of 33.2 outbreaks per 1,000 ship-weeks among 44 Navy ships deployed to the Middle East during a 12-month analysis period (Riddle et al. 2006). It is assumed that norovirus contributed significantly to these outbreaks since a concurrent surveillance study identified norovirus in at least one of the eleven outbreaks included in this study, and in 4 of 4 Navy ships which submitted outbreak stool specimens for testing as part of a separate surveillance report (Riddle et al. 2006)(79 Riddle). Attack rates were similar on large and small military ships (3.3% overall), but larger ships had more frequent outbreaks than smaller ships (66 versus 26 per 1,000 ship weeks, respectively) (Riddle et al. 2006). Smaller ships had increased outbreak durations, possibly because less intense transmission resulted in slower saturation of the susceptible population relative to the more crowded big ships (Riddle et al. 2006). Unlike international cruise ships, which demonstrate a winter-spring predominance, US Navy shipboard outbreaks occurred throughout the year (Riddle et al. 2006).

Norovirus in deployed troops ashore. Norovirus outbreaks among ground troops in deployed settings are also common. Among 20,320 deployed US service members presenting with acute gastroenteritis from 2005-12, 60% of cases were associated with viral pathogens, and norovirus detection increased steadily from 2006 to 2012

("Gastrointestinal Infections, Active Component, U.S. Armed Forces, 2002-2012." 2013). Still another 25,938 cases from this period were documented as "non-specific diarrhea," although norovirus likely contributed significantly ("Gastrointestinal Infections, Active Component, U.S. Armed Forces, 2002-2012." 2013). During Operations Desert Shield and Desert storm, numerous norovirus outbreaks occurred in service members (Hyams et al. 1991; McCarthy, Estes, and Hyams 2000; DeMaio et al. 1993). Norovirus outbreaks also clustered at the beginning of the conflicts in Iraq and Afghanistan (Ahmad 2002; M S Bailey et al. 2008; Centers for Disease Control and Prevention (CDC) 2002; Matson 2005). One norovirus outbreak resulted in the evacuation of 11 British troops from Afghanistan, including one individual with disseminated intravascular coagulation and two requiring ventilator support (Centers for Disease Control and Prevention (CDC) 2002). This outbreak highlighted the potential for norovirus to cause severe disease in individuals under extreme environmental stress (Centers for Disease Control and Prevention (CDC) 2002). In another norovirus outbreak in Iraq, 975 of 1,340 affected British troops were admitted to a field hospital, where significant transmission to medical staff occurred, resulting in hospital closure (M S Bailey et al. 2008; Delacour, Dubrous, and Koeck 2010; Mark S Bailey et al. 2005).

Environmental and behavioral risk factors. The environmental and behavioral risk factors associated with norovirus infection have common themes across populations. In cruise ship patrons, cabin mate sick with diarrhea or vomiting and using vomitus-contaminated bathrooms are associated with infections (Chimonas et

al. 2008). Among infected foodhandlers, the long shedding period of norovirus, particularly in asymptomatically infected or post-symptomatic individuals, contributes to population transmission (M.-A. Sanchez et al. 2017). In military settings, where all population members eat at the same dining facility, norovirus can be introduced by multiple food service workers during outbreaks and then further spread among personnel (Chapman et al. 2011). Additional risks include cleaning bathrooms and exposure to norovirus-contaminated water sources while completing obstacle courses or swimming, and brushing teeth or consuming water that did not come from a water trailer (Chapman et al. 2011). In children living in developing settings, younger age and having animals in the household appears to increase the risk of norovirus infection (BECKER-DREPS et al. 2017). While norovirus is traditionally called the "winter vomiting disease," this seasonality does not appear to be consistent across geographic locations. In Nicaragua, Cameroon, and elsewhere, the rainy season has been associated with increased norovirus incidence among children (BECKER-DREPS et al. 2017; AYUKEKBONG et al. 2014), while in Peru and other tropical countries, norovirus incidence increases during the summer months (Saito et al. 2014). It is not clear whether the "seasonality" of norovirus is due to the effect of environmental factors, such as temperature, humidity, and rainfall, on the virus itself, as suggested by some authors (Shamkhali Chenar and Deng 2016). Alternately, seasonality could be a result of human behaviors that are linked to certain seasons, such as the start of the school year (Kraut et al. 2017), or a combination of the two factors.

NOROVIRUS VACCINES IN THE TROPICS

Recent reviews by Vesikari and colleagues (Vesikari and Blazevic 2015) and Debbink and colleagues (Debbink, Lindesmith, and Baric 2014) detail current vaccine development strategies. Briefly, LigoCyte used a baculovirus-insect cell system to develop the first virus like particle (VLP) vaccine against norovirus GI.1 (El‐Kamary et al. 2010). Their monophosphoryl lipid A (MPL) and chitosan-adjuvanted intranasal vaccine produced a moderate level of protection against the homologous virus in subsequent challenge studies (El‐Kamary et al. 2010; Atmar et al. 2011). This proof of principle vaccine was followed by an MPL-adjuvanted bivalent GI.1/GII.4 VLP vaccine candidate, delivered intramuscularly (Treanor et al. 2014). The corresponding challenge study was performed with a heterologous GII.4 virus, a better representation of natural infection than challenge with a virus homologous to the vaccine VLP (Bernstein et al. 2015). This vaccine provided 100% protection against severe vomiting and severe diarrhea, but was not protective against infection, and only partially protective against symptoms of any severity. Based on a Vesikari scale, the vaccine reduced diarrhea severity significantly, from 7.3 in the placebo group to 4.5 in the vaccine group (Bernstein et al. 2015). Other candidate vaccines in development include a "trivalent" vaccine containing a rotavirus VP6 protein and norovirus GI.3 and GII.4 VLPs (Blazevic et al. 2011; Tamminen et al. 2013); an intranasal dry-powder vaccine (Velasquez et al. 2011); an *Escherichia coli*-produced P particle vaccine (Fang et al. 2013); and a combined norovirus P particle-rotavirus VP8 antigen vaccine (Tan et al. 2011). Multivalent alpha-virus replicon particle platforms for VLP formation (LoBue et al. 2006);

polyvalent norovirus P domain glutathione S-transferase complexes (Wang et al. 2013)**;** and edible vaccine delivery mechanisms (Tacket et al. 2003) are also being explored. Significant work remains to enhance the efficacy of norovirus vaccines against genetically diverse norovirus variants, lengthen the duration of vaccine-induced immunity, lower vaccination costs, determine the acceptability of an adjuvant, and optimize dosing and delivery.

Vaccine efficacy considerations in the tropics. To date, norovirus vaccine trials have occurred in well-nourished adults from high-income countries. However, vaccine underperformance is characteristic of developing settings (von Bubnoff 2011; Qadri et al. 2013; Benjamin A. Lopman et al. 2012), where diarrhea is common, the prevalence of undernutrition is high, and the duration of breastfeeding suboptimal (Haque et al. 2014). Recent mouse model studies demonstrated that malnourished mice infected with murine norovirus develop fairly normal serum antiviral IgG responses, but have significantly reduced mucosal IgA responses that correspond with a lack of protective immunity (Hickman et al. 2014). Parenteral vaccine administration could potentially overcome the apparent intestinal barrier to immunization in undernourished populations. Promoting exclusive breastfeeding and improving nutrition may also improve oral vaccine performance in developing settings (Haque et al. 2014). Given that 70% of pediatric norovirus infections occur between 6 and 24 months of age worldwide, and 60% occur before 12 months of age in high-mortality developing settings, a pediatric norovirus vaccine would have

to be delivered before 6 months of age to prevent the majority of childhood infections (Shioda et al. 2015).

Vaccine cost-effectiveness in the tropics. Using the data collected for the third paper in this dissertation, we worked with Mirelman to develop a model to evaluate norovirus vaccine cost-effectiveness in LMIC populations (Mirelman et al. 2015b). When applied to a hypothetical Peruvian birth cohort, this model found that norovirus vaccination could offer economic value under the right conditions by averting poor health outcomes and substantial healthcare utilization costs (Mirelman et al. 2015b). Assuming a two-dose vaccination cost of $26.44 per individual vaccinated; 85% vaccine coverage; the 47% reported vaccine efficacy in Atmar and colleagues' vaccine trial; and peri-urban diarrhea incidence rates reported by Saito and colleagues, the vaccine cost-effectiveness was $19.86 per diarrhea case averted; $68.23 per outpatient visit averted, and $21,415.95 per disability adjusted life year (DALY) averted (Mirelman et al. 2015b). Using higher norovirus incidence rates from a less developed rural setting, vaccine cost-effectiveness improved to $9.20 per diarrhea case averted; $32.29 per outpatient visit averted; and $10,135 per DALY averted (Mirelman et al. 2015b). This model did not include the indirect costs of norovirus infection or the out-of-pocket direct costs for self-treatment and home care, which are expected to be significant and would further augment the economic value of norovirus vaccination (Rapoport et al. 2014). Likewise, it did not consider the relationship between diarrhea and malnutrition, the prevention of which would result in additional health, social, and

economic benefits. The impact of reduced norovirus transmission to older children and adults was not included in the model, but could be significant since young children play a key role in transmitting norovirus to all age groups (Simmons et al. 2013).

Tallant and colleagues' recently published a new model of diarrhea vaccine cost-effectiveness for deploying US military personnel. Using a diarrhea-based definition of norovirus disease, they calculated a norovirus vaccine cost-effectiveness of $1,344 per duty day lost averted (Tallant et al. 2014a). This model assumed a two-dose vaccination cost of $60.14 per individual vaccinated; 75% vaccine coverage; 80% vaccine effectiveness, reflecting the minimally acceptable military vaccine profile, rather than the efficacy of vaccines currently under development (Riddle and Tribble 2008; Riddle et al. 2008); and a duration of immunity of 3.5 months, which is twice the average duration of US military deployments (Tallant et al. 2014a). The estimated cost per duty day lost averted for military vaccines against *Campylobacter* sp., ETEC, and *Shigella* sp., were $800, $776, and $1,275, respectively (Tallant et al. 2014a). When the norovirus disease definition was modified to include vomiting, the norovirus vaccine cost per duty day lost averted decreased to $572, making it the most cost-effective of the diarrhea vaccines evaluated (Tallant et al. 2014a). As a reference, deployment operational costs per troop were an estimated $935 per day, so a norovirus vaccine with these characteristics would be considered cost-effective for deploying US military personnel (or cost-saving, if vomiting disease is considered) (Tallant et al. 2014a).

Besides young children and travelers (Jennings, Tilley, Ballard, Villanueva, Costa, Lopez, Steinberg, Giannina Luna, et al. 2017), including military service members from high-income countries deployed to tropical regions, other tropical and travel-associated populations that might benefit from norovirus vaccination include the elderly (Kambhampati, Koopmans, and Lopman 2015), hospitalized patients (Kambhampati, Koopmans, and Lopman 2015), individuals with immune compromise, school-aged children, developing country military personnel (Ballard, Saito, et al. 2015a), healthcare workers (Kambhampati, Koopmans, and Lopman 2015), food handlers, food processing facility workers, farm workers, and travel industry workers (Pringle et al. 2015). The key challenge of evaluating norovirus vaccine cost-effectiveness in these populations is the lack of norovirus disease burden data, in addition to uncertainty about the price, dosing, and effectiveness of candidate vaccines. To aid economic evaluations in LMICs, norovirus-specific models should be incorporated into existing cost-effectiveness analysis tools (Jauregui et al. 2015).

CONCLUSIONS

In conclusion, noroviruses are well recognized as the most common cause of acute gastroenteritis in all age groups, and they are becoming the predominant pathogen associated with pediatric diarrhea in the tropics in the wake of the global rotavirus vaccine rollout. In order to appropriately assess disease burden and plan health interventions for populations in developing settings, we will need to refine our case

definitions, severity measures, and intervention assessment tools. We will also need to better elucidate the complex relationship between diarrhea, malnutrition, gut microbiota, environmental enteropathy, enteric co-infections, and the immune system. Multivalent diarrhea vaccines will likely be more cost effective if they are effective against rotavirus, norovirus, and other enteropathogens, such as sapovirus (Oka et al. 2015), which contribute significantly to diarrheal disease. Further, as climate change increases the incidence of diarrhea disease in the tropics, we will need to develop forecasting methods in order to develop appropriate interventions and plan health services (Checkley et al. 2000).

II. FIRST PAPER: EPIDEMIOLOGY AND GENETIC CHARACTERIZATION OF NOROVIRUSES AMONG ADULT PERUVIAN MILITARY PERSONNEL IN AN ENDEMIC SETTING, AMAZON BASIN, 2004-2011

ABSTRACT

Successful vaccination strategies against norovirus will require understanding the burden of disease and relevant genotypes in populations. However, few data are available from cohort studies of adults living in low- and middle-income countries (LMIC). We conducted a nested case-control study within a Peruvian military cohort to characterize the burden of norovirus infection, predominant genotypes, and associated symptoms from 2004 through 2011. Randomly selected case and control stools were tested for norovirus, bacteria, and parasites. The odds ratio of the association between norovirus infection and diarrhea was estimated using multiple logistic regression and co-infection adjusted attributable fractions were calculated. Of the 3,818 cohort study participants, 624 developed diarrhea. Overall and norovirus-associated diarrhea incidence rates were 42.3 and 6.0 per 100 person-years, respectively. The most prevalent norovirus genogroup was GII (72.5%, 29/40), which was associated with diarrhea (AOR 3.4, 95% CI: 1.3-8.7, *P*=0.012). The co-infection adjusted GII attributable fraction was 6.4%. Norovirus was a frequent cause of diarrhea in an adult population followed longitudinally in an LMIC setting. Vaccine strategies should consider targeting adults in endemic settings and special populations that could serve as community transmission sources.

INTRODUCTION

Norovirus, family *Caliciviridae*, causes nearly half of all acute gastroenteritis cases worldwide (Patel et al. 2009). Norovirus infects people of all ages; however, the majority of reported data come from pediatric populations or outbreaks in adults from high-income countries. In adults, norovirus infections have been well described in travelers (Atmar and Estes 2006) and persons working (Ben A. Lopman et al. 2004) or residing in hospitals (Kaufman et al. 2005) military camps (Chapman et al. 2011) aboard ships (Cramer, Gu, and Durbin 2003; Riddle et al. 2004), and in other closed environments (Patel et al. 2009). However, conclusions from these studies may not be generalizable to at-risk adults from low- and middle-income countries (LMIC), where norovirus infections occur at a young age, and reinfection occurs frequently thereafter (Saito et al. 2014).

Noroviruses can be classified into six different phylogenetic clades, or genogroups (GI-GVI) (Glass, Parashar, and Estes 2009). Viruses from GI, GII and, much less frequently, GIV cause human disease. Norovirus genogroups are further classified into genotypes, and genotype GII.4 is further classified into variants (Glass, Parashar, and Estes 2009). The emergence of novel GII.4 variants has initiated all six norovirus-associated acute gastroenteritis pandemics noted since 1995 (Glass, Parashar, and Estes 2009).

Given recent advances in the development of norovirus candidate vaccines, there is a need to estimate the burden of norovirus–associated disease in populations at risk for acute gastroenteritis worldwide, as well as to determine the predominant

norovirus genotypes and their associated pathogenicity (Bernstein et al. 2015; El‐Kamary et al. 2010; Ramani et al. 2017; Blazevic et al. 2011). However, there is limited information about the burden of norovirus-associated adult gastroenteritis in LMIC settings. Therefore, we conducted a nested case-control study within a cohort of Peruvian military personnel in the Amazon Basin to characterize the burden of norovirus infection, predominant genotypes, and associated symptoms in adults followed longitudinally in an LMIC setting.

METHODS

Ethics Statement. This study was approved by the Naval Medical Research Unit No. 6 Institutional Review Board and the military command at Vargas Guerra Army base. Participants provided written informed consent.

Parent cohort study. Between January 1, 2002, and December 31, 2011, the United States Naval Medical Research Unit No. 6 (NAMRU-6) conducted surveillance for acute diarrhea among military personnel at the Vargas Guerra Army base, Iquitos, Peru (Figure 1). Diarrhea was defined as three or more loose or watery stools or two loose or watery stools accompanied by nausea, vomiting, abdominal cramps/pain, or tenesmus within the preceding 24 hours, a modified definition from (Scallan et al. 2004; Majowicz et al. 2008). After at least seven days without diarrhea following enrollment in the cohort study, asymptomatic participants provided basic demographic information and a stool sample ("baseline stool"). Passive surveillance (i.e. recording of persons presenting to the health center) for diarrhea was

conducted until February 1, 2004, after which a study nurse conducted active surveillance, screening each participant daily for diarrhea. If a participant met the case definition, the study nurse recorded the patient's oral temperature, administered a symptom questionnaire, and collected a fresh stool sample. At the conclusion of each diarrhea episode, the study nurse questioned the participant about the episode's impact on daily activities. Impact was classified as mild if, as a result of the episode, the participant experienced no change in daily plans; moderate if the participant experienced an alteration in daily activities but did not miss work; or severe if the participant missed at least part of the work day. Two milliliters of fresh stool were separated for storage at -80^{o}C for future norovirus testing and the remainder sent to the NAMRU-6 laboratory in Iquitos for bacteriological and parasitological evaluation as described below.

Nested case-control study. Within the described cohort, we conducted a nested case-control study using stool samples and data collected from participants who enrolled in the study during the active surveillance period from February 1, 2004, through December 31, 2011. Cases were participants with a first episode of diarrhea, as defined above. Controls were participants who were asymptomatic at the time of stool collection. We randomly selected 200 cases and 200 controls for inclusion in the nested case-control study.

Norovirus detection and sequencing. A suspension of each stored stool specimen (10% wt/vol) was prepared in phosphate buffered saline and RNA extracted using

the Qiagen QIAmp viral RNA kit (Valencia, CA) in accordance with the manufacturer's instructions. Viral RNA was tested for norovirus GI and GII by real-time polymerase chain reaction (PCR) (Apaza et al. 2012). A sample was considered positive if the negative control did not exhibit fluorescent curves. The threshold cycle for the sample was at least 37 for norovirus GI and 39 for GII. Positive samples were then genotyped by sequencing Region C within the capsid gene after conventional PCR and gel-purification of amplicons, comparing results with norovirus prototypic strains using the NoroNet sequence typing tool (Kroneman et al. 2011).

Bacteria and parasite detection. At the time of collection, fresh stool specimens were transported in Cary-Blair medium and wide-mouthed containers to the NAMRU-6 laboratory in Iquitos for bacteria and parasite analysis, respectively. *Escherichia coli* and *Salmonella, Shigella, Yersinia,* and *Campylobacter spp.* were cultured and isolated as previously described (Gray 1995; Nachamkin 1999). Polymerase chain reaction for heat labile and stable enterotoxigenic *E. coli* (ETEC) was performed on five lactose-fermenting colonies morphologically resembling *E. coli* (Stacy-Phipps, Mecca, and Weiss 1995). *Plesiomonas spp.* were identified using the method of Hugh (Hugh 1970). *Vibrio cholerae* were isolated using thiosulfate citrate bile salts sucrose agar (*Laboratory Methods for the Diagnosis of Vibrio Cholerae: Isolation of Vibrio Cholerae from Fecal Specimens* 2016). Microscopy for ova and parasites was performed on saline wet preparations to detect protozoa and

helminth infections. The ether sedimentation technique was used to microscopically identify *Giardia spp* (Ritchie 1948).

Statistical methods. The 2-tailed Student *t* test was used for comparison of continuous outcome variable means. Proportions were compared using Fisher's exact test. The relationship between dichotomous clinical signs and symptoms of gastroenteritis and predictor variables was assessed using univariate logistic regression. A sensitivity analysis was performed by re-classifying the norovirus-bacterial co-infections as norovirus negative and repeating the multivariate logistic regression analysis of the relationship between norovirus infection and diarrheal disease. Norovirus pathogenicity indices (ratio of the prevalence of norovirus in diarrheal stools/prevalence in baseline) were calculated.

The statistical analysis for predictors of diarrhea was performed in several steps; first, the association of diarrhea with specific pathogens was evaluated using simple and multiple logistic regression analyses. Conditional mean imputation was used to handle missing exposure values. Variance inflation factors were all below zero. Forward and backward stepwise selection was used to select variables for regression models. Nested models were compared using likelihood ratio tests and Akaike's Information Criteria. When two nested models were compared using likelihood ratio tests, only the cases common to both regression models following model-wise deletion were used for comparison. Potential interactions were evaluated with regression models. We used adjusted odds ratios and pathogen

prevalence among cases to calculate adjusted population attributable fractions (AFs) to estimate the pathogen-specific disease burden (Bruzzi et al. 1985). The adjusted AF is derived from the logistic regression model that includes other pathogens significantly associated with diarrhea; thus, it is the AF adjusted for the presence of other pathogens. Diarrhea was considered to be attributable to the pathogen identified in stools collected at the time of a diarrhea episode.

For diarrhea incidence calculations in the nested study, the average time to first diarrhea episode was calculated for the randomly selected cases, and average time of observation since enrollment or last diarrhea episode, whichever came first, was calculated for the randomly selected controls. To estimate total person-time observed during the period of active surveillance, we multiplied these numbers by the number of cases and controls, respectively, enrolled during this period. The incidence of diarrhea was then calculated by dividing the number of cases by the total person-time. To calculate norovirus diarrhea incidence, the prevalence of norovirus in stools from cases was multiplied by the diarrhea incidence. Two-sided p-values less than 0.05 were considered statistically significant. STATA version 12.1 (College Station, Texas) (*Stata Data Analysis and Statistical Software* 2015) was employed for all analyses.

RESULTS

Parent cohort study. During the 10-year period of mixed active and passive surveillance, 4,234 males aged 18-34 were enrolled in the parent cohort study (Figure 2). Of these, 643 met the diarrhea case definition.

Nested case-control study. During the 8-year active surveillance period, 3,194 participants were enrolled in the cohort, and 624 developed diarrhea (attack rate 19.5%). The incidence of diarrhea was 42.3 cases per 100 person-years. The 200 randomly selected diarrhea cases and 200 randomly selected controls were roughly evenly distributed over the study period (data not shown). Of these, 184 case and 176 control stools were available for norovirus analysis. Norovirus was identified in 14.1% (26/184) and 8.0% (14/176) of stool samples from cases and controls, respectively (Table 1). The norovirus-associated diarrhea attack rate was 2.7% (14.1% prevalence of norovirus in case stools * 19.5% diarrhea attack rate) with an incidence of 6.0 norovirus diarrhea cases per 100 person-years. The norovirus pathogenicity indices were 1.4 for GI, 2.1 for GII, and 1.8 overall.

Diarrhea cases had a higher prevalence of ETEC (P=0.001) and *Shigella* spp. (P<0.001) relative to controls, but a lower prevalence of *Trichuris* (P=0.006) (Table 1, S1 Dataset). Multiple logistic regression analysis revealed norovirus infection to be significantly associated with diarrhea (AOR 2.8, 95% CI: 1.3-6.0, P=0.008). However, when stratified by genogroup, this relationship only remained significant for norovirus GII (P=0.012). The norovirus GI variable was subsequently dropped from the adjusted model (Table 2). Other variables associated with diarrhea in the

adjusted norovirus GII model were infection with *Shigella* spp. (*P*<0.001), ETEC (*P*=0.004), and *Trichuris* (*P*=0.005), the latter being associated with a decreased risk of diarrhea. After reclassifying norovirus-bacteria co-infected individuals as norovirus-negative in the sensitivity analysis, the relationship between GII infection and diarrhea remained statistically significant in the adjusted multiple logistic regression model (AOR 2.7, CI: 1.0-7.1, *P*=0.047). The AFs for norovirus GII, *Shigella* spp., and ETEC were 6.4% (95% CI: 1.0-9.0%), 18.2% (95% CI: 14.3-20.0%), and 7.1% (95% CI: 3.8-7.9%), respectively.

Of all noroviruses identified, 30.0% (12/40) were GI, 72.5% (29/40) were GII, and 2.5% (1/40) were mixed GI/GII infections. Twenty-five (61.0%) of the 41 norovirus isolates could be genotyped (18 from cases and 7 from controls), of which 11 were norovirus GI and 14 were GII (Table 3). The predominant norovirus GI and GII genotypes were GI.4 (7 stools) and GII.4 (6 stools).

Among all norovirus-infected participants, co-infection with norovirus and one or more organisms was present in 67.5% (27/40), including 12.5% (5/40) of participants infected with norovirus and three other organisms. Norovirus co-infection was most often with chronically carried soil-transmitted helminths (n=9), *Giardia* (n=5), or both (n=2), although co-infection with norovirus and either *Shigella* (n=4) or ETEC (n=4) was also noted (Table 4). *Trichuris* was less frequently found in cases (15.3%, 30/196) relative to controls (16.8%, 52/194) (*P*=0.006). However, among diarrhea cases, *Trichuris* was more frequent in GII-positive stools

(30.0%, 6/20) relative to GII-negative ones (12.4%, 20/161) (P=0.046). No other statistically significant associations were noted.

Of norovirus positive cases, 23.5% (6/26) reported that their symptoms had minimal impact on their daily activities, 73.1% (19/26) reported a moderate impact, and 3.8% (1/26) reported a severe impact. None of the participants were hospitalized and there were no deaths. There was no significant difference in the frequency of clinical symptoms associated with norovirus diarrhea compared to other etiologies. Infection with norovirus GII produced more severe disease than norovirus GI, reaching statistical significance for abdominal pain (94.7%, 18/19 versus 50.0%, 3/6, P=0.031) (Table 5).

DISCUSSION

Norovirus infection was the second most common diarrheagenic pathogen detected in our study. The virus, especially norovirus GI, was also frequently present in stools of persons who had not recently experienced diarrhea, as has been frequently reported (Lindesmith et al. 2008). Furthermore, co-infection with norovirus and other enteric organisms was frequent. In this pathogen diverse setting it can be difficult to determine the specific etiologic agent of a case of diarrhea. Nevertheless, the pathogenicity index of norovirus, particularly the GII genogroup, was high relative to other major enteric pathogens. Norovirus was the sole pathogen in 27.0% of norovirus-infected diarrhea cases and 69.2% of norovirus diarrheal cases did not have a co-infecting bacterial pathogen. Together, these observations suggest that

norovirus is the cause of the majority of the diarrheal illness with which it is associated and a major cause of diarrhea in the study population. Possible reasons for the presence of norovirus in baseline stools include continued shedding in persons recovering from a bout of diarrhea experienced before the wash-out period (norovirus, particularly GII, can be shed for weeks to months after an episode of diarrhea) (Atmar and Estes 2006; Saito et al. 2014), asymptomatic infection due to pre-existing innate or acquired immunity, and insufficient infectious dose.

Few studies have described the disease burden of norovirus in adults in LMIC settings. The burden of acute diarrhea attributable to norovirus noted in our study is similar to that noted in children in Peru (Saito et al. 2014; Pablo Peñataro Yori et al. 2009), although the risk of infection in our closed population on a military base is likely higher than that typically noted in adults in the community due to the military base's relatively increased population density, poor sanitation and hygiene, and the resultant increased burden of norovirus contamination of the environment. Interestingly, in a rural community-based study of norovirus by Peñataro Yori *et al* in children in the same region as our study site, the proportion of diarrheal cases attributable to norovirus was highly age dependent, accounting for 22-25% in children 0-2 years old but steadily falling with age, accounting for less than 5% by age 4-5 years (Pablo Peñataro Yori et al. 2009). The authors concluded that naturally acquired immunity protected children as they age. However, our results suggest that many adults remain susceptible to infection and disease from norovirus. We cannot determine whether the cases of norovirus diarrhea in our

study resulted from viral evolution for immune escape or first infection in naïve hosts, although given that norovirus infection in children is nearly universal in Peru (Saito et al. 2014), we highly suspect the former. The specific norovirus genotypes circulating at the time of each study may also play a role, as immunity is likely genotype-specific (Patel et al. 2009). Norovirus genotypes were not determined in the Peñataro Yori *et al* study. Another possible factor that could contribute to variable susceptibility of adults to norovirus-induced diarrhea, not determined in either study, is histo-blood antigen group, which appears to mediate norovirus-binding through epithelial cell surface ligands (Lindesmith et al. 2008). It is also worth noting that Yori *et al* reported fever in 71.4% of children with norovirus-associated diarrhea versus none of the adults in our study, suggesting that at least some clinical features of norovirus infection may be age dependent, or perhaps change with successive norovirus infections.

A successful norovirus vaccine will need to provide protection against various norovirus genogroups and types (Daniel C. Payne et al. 2013). Our investigation provides detailed data on the norovirus genotypes circulating in the study region. As with many other studies, we found norovirus GII to be a prevalent and particularly pathogenic genotype (especially GII.4), suggesting that it should be at least one major target of a norovirus vaccine. Similar findings have been reported in Brazil, Argentina, and Bolivia (Blanco Fernández et al. 2011; Ferreira et al. 2010; McAtee et al. 2016). Previous research has shown that, compared with persons infected with other norovirus genotypes, those infected with norovirus GII.4 shed the virus in

larger numbers (Lee et al. 2007), transmit the virus at higher rates (Lee et al. 2007), and have poorer outcomes (Desai et al. 2012). Further investigation of the noroviruses that we were unable to genotype will be important to fully understand the epidemiology and the organisms and epitopes relevant to a successful vaccine.

The range of organisms and co-infections noted in our study is similar to that previously reported in both children and adults in the region (Pablo Peñataro Yori et al. 2009; Jones et al. 2004). Interestingly, co-infection with *Trichuris* was associated with a decreased risk of diarrhea in persons also infected with norovirus. *Trichuris* co-infection in children has been documented with numerous enteric organisms, including *Entamoeba histolytica* (Jung and Beaver 1951), *Shigella* spp. (Gilman, Davis, and Fitzgerald 1976), *Salmonella* spp. (Gilman, Davis, and Fitzgerald 1976), and a variety of helminths (Lello et al. 2013). We speculate that *Trichuris* infection in our population serves as a proxy for frequent previous infection and consequent enhanced immunity to enteric pathogens, including noroviruses. The apparent lack of a protective effect of *Trichuris* infection against norovirus GII infection relative to GI infection may relate to the increased mutation rate and thus genetic variability of the norovirus GII genogroup, allowing these viruses to present varied epitopes that escape immune system detection (Glass, Parashar, and Estes 2009).

Limitations to our study include the 1) Lack of a validated diarrhea severity score for adults, which limited our ability to evaluate the relationship between norovirus

infection and disease severity; 2) Use of a case definition that did not include vomiting without diarrhea, which is common with norovirus infection, which could result in underestimation of the contribution of norovirus to gastroenteritis in the study population; 3) Short (1-week) washout period before baseline stool evaluation, which could result in underestimation of the norovirus pathogenicity index, given that norovirus may be shed for a median of 28 days in adults following acute infection (Atmar et al. 2008); 4) Use of qualitative rather than quantitative PCR, precluding exploration of possible associations between viral load and disease; 5) Use of conventional PCR, which is less sensitive than rRT-PCR, to obtain material for genetic sequencing, which could have biased detection toward norovirus genotypes and variants present in higher viral loads (Table 3). Nor would our methodology detect norovirus GIV; 6) Lack of data on the precise duration of each participant on the base, which may have led to overestimation of the person-time at risk and an underestimation of incidence rates; and 7) Small sample size, which often limited statistical analysis of the temporal distribution and disease associations of specific norovirus genotypes and variants. This latter point is especially important considering that norovirus pandemics featuring novel variants occur every two to four years (Glass, Parashar, and Estes 2009). Rapid norovirus mutation and recombination events may result in the development of novel norovirus genotypes and variants, particularly in the GII genogroup, in a manner similar to that of the influenza A virus (Lam et al. 2012). In settings where norovirus is prevalent, mutations may occur at an accelerated pace, leading to the emergence of new variants capable of causing community epidemics.

In conclusion, our study helps extend knowledge of the complex epidemiology of adult norovirus infection and disease in a closed setting in a LMIC. Such populations, which are traditionally prone to norovirus outbreaks with high transmission rates, could serve as potential sources of introduction into the community and thus must be considered in future control and vaccination strategies.

III. SECOND PAPER: APPLICATION OF A RELATIVE COST-EFFECTIVENESS ANALYSIS MODEL TO THE EVALUATION OF PATHOGEN-SPECIFIC VACCINES AGAINST *CAMPYLOBACTER,* ETEC, SHIGELLA, AND NOROVIRUS TO A PERUVIAN ADULT MILITARY POPULATION IN THE AMAZON BASIN

ABSTRACT

Adult populations in low- and middle-income countries (LMIC) are often neglected during the vaccine implementation planning process. We adapted an economic model developed by the United States Department of Defense (DoD) to evaluate the cost-effectiveness of a vaccine acquisition strategy and modified for *Campylobacter*-, ETEC-, *Shigella*-, and norovirus-associated gastroenteritis. We compared the cost-effectiveness of vaccine implementation with current medical management in the Peruvian military, a special population of LMIC adults with a high incidence of infectious diarrhea. Using US$ per Duty Day Lost as an outcome measure, we found that, relative to implementation in the United States, *Shigella* vaccine implementation is significantly more cost-effective for the Peruvian military (CER_{DDL}=$350 versus $1275), and norovirus vaccine implementation is moderately more cost effective (CER_{DDL}=$926 versus $1344). These data suggest that future pathogen-specific gastroenteritis vaccine acquisition strategies for the Peruvian military may consider protecting troops against these pathogens.

INTRODUCTION

While frequently discussed in the context of child mortality in low- and middle-income countries (LMIC), acute gastroenteritis also contributes significantly to adult disease in these settings (Ballard, Reaves, et al. 2015a). In Peru, the incidence of acute gastroenteritis among military troops deployed to the Amazon Basin was 42.3

per 100 person-years (Ballard, Reaves, et al. 2015a), while the Peruvian military's electronic syndromic surveillance system identified 21.5 cases of acute gastroenteritis per 100 person-years for adult active duty and reserve service members, dependents, and civilian employees in garrison. Among Peruvian troops deployed to the Amazon Basin (Alsentzer et al. 2014), the most frequently isolated non-parasitic gastroenteritis-associated pathogens were *Shigella* (22%), norovirus (14%), enterotoxigenic *Escherichia coli* (ETEC) (8%), *Salmonella* (2%), *Plesiomonas* (1%), *Vibrio* (0.5%), and *Campylobacter* (0.5%) (Ballard, Reaves, et al. 2015a). Vaccines against specific gastroenteritis-associated pathogens are rapidly approaching the market. Preliminary analysis indicates potential benefit for pediatric populations in LMIC settings (Mirelman et al. 2015b), in addition to adult travelers (Ballard, Saito, et al. 2015a; Jennings, Tilley, Ballard, Villanueva, Costa, Lopez, Steinberg, Giannina Luna, et al. 2017) and military personnel from high-income countries (Tallant et al. 2014a). However, the potential use of these vaccines in adult populations in LMIC settings has not been well characterized. Here, we calculate the cost-effectiveness of implementing vaccines currently under development for *Campylobacter*, ETEC, *Shigella*, and norovirus in a military population in a low- and middle-income country setting.

METHODS

Model overview. We adapted an economic model developed by the United States Department of Defense (DoD) to evaluate the cost-effectiveness of a vaccine acquisition strategy and modified for *Campylobacter*-, ETEC-, *Shigella*-, and

norovirus-associated gastroenteritis (Tallant et al. 2014a; Riddle et al. 2008). We compared the cost-effectiveness of vaccine implementation with current medical management in the Peruvian military. In the model, vaccination could result in susceptibility to specific pathogen-associated gastroenteritis or protection. Susceptible individuals had two possible outcomes upon infection: symptomatic gastroenteritis or asymptomatic infection (Tallant et al. 2014a). Symptomatic individuals might seek or not seek medical evaluation, and those seeking evaluation could be managed in three potential ways: no treatment, treatment provided by a health-care provider, or aeromedical evacuation for severe disease manifestations (Tallant et al. 2014a).

We assumed that the Peruvian military would not be responsible for research and development associated with vaccines against enteric diseases. Similar to the DoD model, we assumed a one-year time horizon, discounting costs to account for future inflation. We assumed that vaccine administration would take place before each deployment due to waning immunity. Pathogen-specific enteric vaccine-associated costs included: purchase price of a two-dose vaccine; administrative costs, such as storage, logistics, delivery, and monitoring; and adverse event treatment costs. We used previously published Peruvian vaccination program costs for these estimates (A. D. Clark et al. 2009). We assumed that these vaccines would not be purchased unless they met minimal military parameter requirements for efficacy of 80%. The target Peruvian military population size was 120,660, consistent with published force strength estimates ("Global Fire Power" 2016). We used a deployment

duration of 3.5 months, consistent with mission estimates provided by the Peruvian military liaison on the Norovirus Working Group. We appreciated all costs to their current 2013 value using the Consumer Price Index.

Estimates of norovirus-specific probability estimates, pathogen incidence, and costs for the Peruvian military **(Table 6).** To adapt the model to the Peruvian military population, we obtained probability estimates for current gastroenteritis management approaches, military healthcare provider treatment types, and pathogen-specific gastroenteritis prevalence estimates from surveillance data published by our working group (Ballard, Reaves, et al. 2015a). We obtained aeromedical evacuation and treatment cost data from the Peruvian military medical liaison on the working group. As none of these pathogen-specific vaccines with the desired parameters are currently on the market, we estimated vaccine costs for the Peruvian military using 2008 Pan American Health Organization revolving fund prices for Rotarix (A. D. Clark et al. 2009; "2008 PAHO Revolving Fund Vaccine Prices" 2008), which match pre-2016 GAVI pricing ("GAVI Alliance Secures Lower Price for Rotavirus Vaccine" 2012). We converted cost estimates in Peruvian currency to USD using an exchange rate of 0.31 USD per Peruvian sol.

Outcome measures and sensitivity analysis. We selected duty days lost (DDL) due to norovirus-associated gastroenteritis as the outcome measure. The cost-effectiveness ratio (CER) numerator was the cost of administering the vaccine to the Peruvian military population minus the expected care costs in the absence of

vaccine administration. The CER denominator was the DDL averted by incorporating pathogen-specific vaccine administration into the Peruvian military's pre-deployment vaccine program. We used DDL estimates determined by a previously published literature review and data collected from DoD surveillance among US military recruits seeking care for moderate to severe gastroenteritis (Tallant et al. 2014a).

To determine which model parameters have the greatest impact on the CER for each pathogen, we performed a one-way sensitivity analysis using high and low values for each parameter. We compared the resultant CERs to base case estimates to identify high-impact variables.

RESULTS

We present the base case results in Table 7. Implementation of a vaccine against *Campylobacter*, ETEC, *Shigella*, or norovirus by the Peruvian military would potentially prevent 137, 2223, 5902, or 3870 cases, respectively, of gastroenteritis annually. The total annual cost of the program would be $1,447,920 at $15 per vaccine series administered. Following vaccine implementation, the number of *Campylobacter*-, ETEC-, *Shigella*-, and norovirus-associated DDL is projected to decrease from 116, 1201, 4699, and 1839, respectively, to 23, 240, 940, and 368, respectively, per year. The predicted reduction in total annual cost of care following vaccine introduction is from $3622, $58681, $155758, and $102148, respectively, to $543, $8802, $23364, and $15322, respectively. Based on the input parameters

described, pathogen-specific vaccine implementation would result in a respective CER per DDL (CER_{DDL}), or cost per duty day lost averted, of $15598, $1456, $350, CEand $926, and a respective CER per diarrhea day averted of $2418, $246, $60, and $229 for *Campylobacter*, ETEC, *Shigella*, and norovirus.

We display the one-way sensitivity analysis results in tornado diagrams for each pathogen (Figure 4) and demonstrate that 48-49% of the variation in the economic models for each pathogen can be accounted for by the relationship between deployment duration and CER_{DDL}. For all four pathogens, the most influential input parameters in this model (in order of importance) include: deployment duration, monthly incidence of pathogen-associated disease, pathogen prevalence, vaccine efficacy, vaccine coverage, and cost per dose of the vaccine. Together, all of these input variables account for 86-88% of the variance in the model, depending on the pathogen.

DISCUSSION

In Peruvian military populations deploying to the Amazon Basin, vaccines against *Shigella* appear to be the most cost-effective in preventing duty days lost to diarrhea, followed by vaccines against norovirus, ETEC, and *Campylobacter.* Compared to estimates of pathogen-specific CER_{DDL} for vaccines implemented in military populations from high-income countries, such as the United States, *Shigella* vaccine implementation is significantly more cost-effective for the Peruvian military (CER_{DDL}=$350 versus $1275), and norovirus vaccine implementation is moderately

more cost effective (CER_{DDL}=$926 versus $1344) (Tallant et al. 2014a; Riddle et al. 2008). Neither *Campylobacter* (CER_{DDL}= $15598 versus $800) nor ETEC ($CER_{DDL}$=$1456 versus $776) vaccine implementation is more cost-effective in the Peruvian military population relative to that of the United States (Riddle et al. 2008). These data suggest that future vaccine acquisition strategies for the Peruvian military should focus on vaccines against *Shigella* and norovirus.

Many cost-effectiveness analyses use quality adjusted life years (QALYs) to estimate effectiveness. However, given the self-limiting nature of most acute gastroenteritis, we deemed DDL more appropriate effectiveness measure than QALYs because it more accurately represents the impact of gastroenteritis on the military population and can be better understood by commanders and medical planners. At present, no established cutoff exits for the acceptable dollar value deemed to be cost-effective for the DDL averted outcome. The value of a DDL averted may vary based on the intensity, stability, and location of operations underway when an outbreak occurs. In the United States military, planners budget an operation cost per troop of $28,057 (2012 dollars) per month, or $935 per day (Tallant et al. 2014a). Previous estimates have exceeded this at $2000-$3000 per troop per day. In low- and middle-income country settings, this value is likely much lower, although not well characterized in the literature (Tallant et al. 2014a). Further, the distraction and discomfort of gastrointestinal illness is associated with indirect costs that are not easily calculated.

Estimating vaccine costs for military populations in low- and middle-income countries is complex. Another VLP-based vaccine, human papilloma virus (HPV), from PAHO's Revolving fund and from a study in Brazil (Mirelman et al. 2015b) estimates the vaccine cost at $26.44 per series, while GAVI provides vaccination for $4.50 USD per series. However, using a potential norovirus vaccine in a military population may eliminate the opportunity for the Peruvian government to receive subsidized pricing for the vaccine, which may be exacerbated further by the recategorization of Peru by World Bank as an upper middle-income country, as opposed to its previous designation of lower middle income.

The simplicity of the model limited our evaluation of several factors, including the potential for herd immunity to reduce or significantly disrupt norovirus transmission during outbreaks and residual effects on transmission of norovirus after troops return to garrison (ie. when they aren't deployed on a mission). Additionally, this model did not consider the potential reduction in chronic health sequellae, such as post-infectious irritable bowel syndrome, that might contribute to a decrease in future medical costs for this population.

In conclusion, vaccine acquisition strategies for the Peruvian military should focus on vaccines against *Shigella* and norovirus. Cost-effectiveness ratios per duty day lost averted should be further defined for specific military populations and operations in order to improve decision-making for operational planners.

IV. THIRD PAPER: ETIOLOGY OF MEDICALLY-ATTENDED GASTROENTERITIS IN CHILDREN YOUNGER THAN FIVE YEARS OF AGE FOLLOWING UNIVERSAL ROTAVIRUS VACCINE IMPLEMENTATION IN PERU

ABSTRACT

Future pediatric gastroenteritis control strategies require understanding the shifting enteropathogen landscape following Rotarix™ vaccine implementation in developing settings. We conducted this case-control study of children younger than five years of age with and without gastroenteritis at the national children's hospital in Peru following universal Rotarix™ vaccine implementation. We tested case and control stools for viruses, bacteria, and parasites. and calculated coinfection-adjusted attributable fractions (AFs) to determine the burden of gastroenteritis attributable to specific pathogens. The following six pathogens were independently positively associated with gastroenteritis: norovirus GII (AF 29.1, 95% CI: 28.0-32.3); rotavirus (AF 8.9, 95% CI: 6.8-9.7); sapovirus (AF 6.3, 95% CI: 4.3-7.4); ETEC St=/Lt+St+ (AF 2.4, 95% CI: 0.6-3.1); *Shigella* (AF 2.0, 95% CI: 0.4-2.2); and astrovirus (AF 2.8, 95% CI: 0.0-4.0). Caliciviruses are an important cause of diarrhea among children with medically-attended diarrhea in Peru. Control strategies should consider multivalent vaccines that target these pathogens.

INTRODUCTION

Worldwide, diarrhea is a leading killer of children younger than five years of age. Prior to the introduction of universal national vaccination programs, rotavirus was the major viral pathogen causing diarrhea-associated deaths. In countries, such as Peru, where Rotarix™ has been implemented with high coverage rates, caliciviruses,

specifically norovirus and sapovirus, have emerged as important diarrhea-associated pathogens among children in the community setting. However, little is known about the relative contribution of these and other pathogens to severe diarrhea following rotavirus vaccine implementation in developing settings.

We conducted this case control study in the national children's hospital in Peru following the universal implementation of a vaccine against rotavirus in order to a) determine the pathogen-specific attributable risk among children with medically-attended gastroenteritis; b) evaluate the relationship between infection with specific pathogens and gastroenteritis severity; and c) describe the genetic diversity of circulating norovirus, sapovirus, and rotavirus to inform prevention and control measures.

METHODS

Ethics statement. The institutional review boards of Naval Medical Research Unit No. 6, Instituto Nacional de Salud del Niño, Universidad Peruana Cayetano Heredia, and Asociación Benéfica PRISMA approved this study. Legal authorized representatives of children provided written informed consent for participation at the time of enrollment.

Study population. Between 31 October 2013 and 31 May 2015, we prospectively enrolled children younger than five years of age accessing care at the national children's hospital in Lima, Peru, for acute gastroenteritis (cases) or reasons other

than gastroenteritis (hospital-based controls). We recruited community-based controls from an ongoing cohort study in a peri-urban shantytown in southern Lima (Saito et al. 2014). We defined acute gastroenteritis as the onset of diarrhea or vomiting within three days of enrollment ("WHO Initiative to Estimate the Global Burden of Foodborne Diseases" 2017). We defined a diarrheal episode by the presence of ≥3 liquid or semiliquid stools in 24 hours. For infants younger than 2 months, we based the definition on the caretaker's assessment that the child had diarrhea (Saito et al. 2014). An episode ended when the child had two consecutive days without diarrhea. "Pathogen-associated gastroenteritis" or symptomatic infection was defined as positive identification of a specific pathogen in a stool sample collected during the first three days of the gastroenteritis episode. "Asymptomatic" infection occurred when no symptoms were reported within seven days of the positive specimen collected at the time of enrollment. Exclusion criteria were: hospitalization for ≥1 month at birth, any congenital defect, birth weight <1500 g, and a diarrhea episode within the 30 days preceding enrollment. At the time of enrollment, a trained healthcare provider administered a survey to assess each participant's demographics, socioeconomic status, and history of present illness. Study nurses collected the participant's anthropometric data, and a study physician conducted a clinical examination before collecting serum, stool (whole stool or rectal swab), and saliva samples. After enrollment, a study nurse called each participant's caregiver daily to prospectively collect information about the clinical features of illness, health-related behaviors, and costs related to the illness.

These daily follow up phone calls continued until the caregiver reported that the participant was asymptomatic for 48 hours.

Calicivirus detection and genotyping. A suspension of each stool specimen (10% wt/vol), stored at -80 °C, prepared in phosphate buffered saline, was allowed to thaw at 4 °C, and RNA was extracted using the Qiagen QIAmp viral RNA kit (Valencia, CA) in accordance with the manufacturer's instructions. Viral RNA was tested for norovirus GI and GII by real-time polymerase chain reaction (PCR) (Apaza et al. 2012). A sample was considered positive if the negative control did not exhibit fluorescent curves. The threshold cycle for the sample was at least 37 for norovirus GI and 39 for GII. Positive samples were then genotyped by sequencing Region C within the capsid gene after conventional PCR and gel-purification of amplicons, comparing results with norovirus prototypic strains using the NoroNet sequence typing tool (Kroneman et al. 2011). We detected and genotyped sapovirus as described in (X. Liu et al. 2016). Aichi virus and astrovirus were identified on a randomly selected subset of children using conventional methods (Chuchaona et al. 2017; Lopez et al. 2017).

Rotavirus detection and genotyping. To identify rotavirus-positive samples, we conducted qRT-PCR on the diluted the thawed stool specimen suspensions, targeting an 87 bp fragment of the highly conserved NSP3 region of rotavirus group A using previously described primers and protocols from the Centers for Disease Control and Prevention, Atlanta, GA (Freeman et al. 2008). We genotyped all

rotavirus positive samples using a semi-nested RT-PCR to identify VP7 (G) and VP4 (P) genotypes as described previously (Gouvea et al. 1990; Gouvea and Santos 1999; Das et al. 1994).

Bacteria and parasite identification. At the time of collection, fresh stool specimens were transported in Cary-Blair medium and wide-mouthed containers to the NAMRU-6 laboratory in Iquitos for bacteria and parasite analysis, respectively. *Escherichia coli* and *Salmonella, Shigella, Yersinia,* and *Campylobacter spp.* were cultured and isolated as previously described (Gray 1995; Nachamkin 1999). Polymerase chain reaction for heat labile and stable enterotoxigenic *E. coli* (ETEC), diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and Shigatoxin 1- and Shigatoxin 2-producing shigatoxigenic *E. coli* (STX1/STX2 STEC) was performed on five lactose-fermenting colonies morphologically resembling *E. coli* (Stacy-Phipps, Mecca, and Weiss 1995). *Plesiomonas spp.* were identified using the method of Hugh (Hugh 1970). *Vibrio cholerae* were isolated using thiosulfate citrate bile salts sucrose agar (*Laboratory Methods for the Diagnosis of Vibrio Cholerae: Isolation of Vibrio Cholerae from Fecal Specimens* 2016). Microscopy for ova and parasites was performed on saline wet preparations to detect protozoa and helminth infections. We used the ether sedimentation technique to microscopically identify *Giardia spp* (Ritchie 1948).

Statistical methods. Statisical analysis was performed using Stata version 13.0 (StataCorp LP, College Station, TX). By convention, *P* values less than 0.05 were considered statistically significant. Exploratory analysis of participant demographics, diarrhea risk factors, and clinical features of illness was performed, and child growth parameters were calculated using the World Health Organization standards (De Onis et al. 2017). The 2-tailed Student *t* test was used for comparison of continuous outcome variable means. Proportions were compared using Fisher's exact test.

The statistical analysis for predictors of diarrhea was performed in several steps; first, the association of diarrhea with specific pathogens was evaluated using simple and multiple logistic regression analyses. Variance inflation factors were all below zero. Forward and backward stepwise selection was used to select variables for regression models. Nested models were compared using Akaike's Information Criteria. Potential interactions were evaluated with regression models. We performed sensitivity analyses to evaluate the relationship between viral infections and gastroenteritis by recategorizing virus-bacteria coinfected children as positive for only the coinfecting bacteria and reevaluating the relationship between specific viruses and gastroenteritis using the logistic regression analysis described above. We used adjusted odds ratios and pathogen prevalence among cases to calculate adjusted population attributable fractions (AFs) to estimate the pathogen-specific disease burden (Bruzzi et al. 1985). The adjusted AF is derived from the logistic regression model that includes other pathogens significantly associated with

diarrhea; thus, it is the AF adjusted for the presence of other pathogens. Diarrhea was considered to be attributable to the pathogen identified in stools collected at the time of a diarrhea episode.

RESULTS

Participant characteristics (Table 8)***.*** During the 18-month study period, we enrolled 1,788 participants (57% male) in the study: 932 medically-attended children with gastroenteritis and 856 controls without gastroenteritis. Of the cases, 666 had both diarrhea and vomiting, and 164 had only diarrhea, and 102 had only vomiting. Of the controls, 400 were hospital-based, and 456 were community-based. Relative to controls, case enrollment was significantly higher from December through May. Cases scored significantly higher than controls on the modified PPI scale (39 v. 32) and were significantly more likely than controls to have improved sanitation (94% v. 40%) and water sources (90% v. 41%) at home. Cases and controls had similar blood group and Rhesis factor prevalences. Cases had significantly higher first (97% v. 92%) and second (92% v. 85%) Rotarix™ immunization rates relative to controls. Cases had significantly lower length-for-age (0.05 [SD 2.28] v. 0.31 [SD 1.81]_ and weight-for-age 90.11 [SD 1.28] v. 0.53 [SD 1.23]) z scores relative to controls, in addition to having lower hematocrit levels relative to controls (35.0 v. 35.5).

Pathogen-specific gastroenteritis disease burden (Table 8, 9)***.*** We identified at least one organism in 73% of case stools and 44% of control stools. Among case

stools, we identified viruses in 57%, bacteria in 39%, and parasites in 5%. Among control stools, we identified viruses in 21%, bacteria in 26%, and parasites in 15%. We identified two or more organisms in 34% of cases (215 cases had two, 48 had three, 5 had four, and 2 had five), and 4% of controls (32 had two, and one had four). The most prevalent viruses and bacteria among cases were: norovirus (36%), DAEC (16%), rotavirus (11%), sapovirus (8%), EAEC (7%), ETEC (5%), astrovirus (5%), EPEC (5%), *Campylobacter* sp. (3%), *Aeromonas* (3%), *Shigella* sp. (2%), *Salmonella* (2%), and STEC (1%). Among controls, the most prevalent were: DAEC (13%), norovirus (12%), EPEC (4%), EAEC (4%), rotavirus (3%), sapovirus (3%), astrovirus (3%), ETEC (3%), *Salmonella* (2%), *Aeromonas* (2%), and *Campylobacter* sp. (2%). The remaining pathogens were absent or less than 1% prevalent. The most prevalent parasites among cases were *Giardia* (3%), *Cyclospora* (0.9%), *Entamoeba coli* (0.7%), *Chilomastix mesnili* (0.7%). The most prevalent among controls were *Giardia* (9%), *Entamoeba coli* (5%), *Endolimax nana* (2%), *Chilomastix mesnili* (1%), and Cryptosporidium (0.6%). The remaining pathogens were absent or less than 0.5% prevalent.

Relative to those of controls, stools from children with gastroenteritis had a significantly higher prevalence of norovirus (36% v. 12%, pathogenicity index [PI]=2.9), rotavirus (11% v. 3%, PI=3.1), sapovirus (8% v. 3%, PI=2.8), EAEC (7% v. 4%, PI=1.9), ETEC (5% v. 3%, PI=2.0), astrovirus (5% v. 3%, PI=2.9), *Campylobacter* sp. (3% v. 2%, PI=1.9), and *Shigella* sp. (2% v. 0.2%, PI=8.9). Relative to those of cases, stools from asymptomatic controls had significantly higher prevalences of

Giardia (9% v. 3%), *Entamoeba coli* (5% v. 1%), and *Endolimax nana* (2% v. 0%). After adjusting for age, sex, and socioeconomic status, multiple logistic regression analysis revealed the following were significantly positively associated with acute gastroenteritis: *Shigella* sp. (AOR 9.1, 95% Confidence Interval [CI]: 1.9-43.0, *P*=0.005); norovirus GII (AOR 6.4, 95% CI: 4.4-9.4, P<0.001); rotavirus (AOR 6.0, 95% CI: 2.8-12.6, *P*<0.001); sapovirus (AOR 3.6, 95% CI: 2.0-6.6, *P*<0.001); ETEC with LT/ST or ST only (AOR: 3.1, 95% CI: 1.2-8.2, *P*=0.021); and astrovirus (AOR 2.2, 95% CI: 1.0-4.4, *P*=0.038) (Table 9). The presence of any parasite was protective for acute gastroenteritis (AOR 0.3, 95% CI: 0.2-0.5, *P*<0.001). After reclassifying the 152/433 norovirus-, 41/127 rotavirus-, 47/104 sapovirus-, and 31/37 astrovirus-bacteria coinfected individuals as negative for these respective viruses in the sensitivity analysis, norovirus, rotavirus, and sapovirus all remained significantly associated with gastroenteritis with AORs 3, while astrovirus did not remain significantly associated with gastroenteritis (*P*=0.233). Of the 31 astrovirus-bacteria coinfected individuals, 2 were coinfected with an St+ ETEC, the remaining 29 were not infected with a gastroenteritis-associated bacteria. When only the St+ ETEC was considered astrovirus-negative in the sensitivity analysis, the relationship between astrovirus and gastroenteritis trended back toward significance (*P*=0.062). The AFs for *Shigella* sp., norovirus GII, rotavirus, sapovirus, St+/Lt+St+ ETEC, and astrovirus were 2.0 (95% CI: 0.4-2.2); 29.1 (95% CI: 28.0-32.3); 8.9 (695% CI; 6.8-9.7); 6.3 (95% CI: 4.3-7.4); 2.4 (95% CI: 0.6-3.1); and 2.8 (95% CI: 0.0-4.0), respectively.

Clinical features and severity of gastroenteritis (Table 10)***.*** Of the 932 cases, the mean score (SD) was 12.1 (2.3) on the Vesikari severity scale and 11.5 (2.2) on the Clark severity scale. Although the scoring systems were not identical, scales had similar trends with regard to the relative mean severity score of specific pathogens. Of the 932 gastroenteritis cases, 701 (75%) were defined as severe by the Vesikari scale (20 points total; <11 nonsevere; ≥11 severe) versus 9 (<1%) by the Clark scale (24 points total; ≤4 poin; 9-16 moderate; 16 severe) (*P*<0.001). All of the cases defined as severe by the Clark scale were also defined as severe by the Vesikari scale. However, only 9 (1%) of the Vesikari severe cases were defined as severe by the Clark scale, while 653 (93%) and 40 (6%)were defined as moderate and mild, respectively. The mean Vesikari (12.7 v. 11.8, P<0.001) and Clark (11.7 v. 11.4, P=0.016) scores were significantly higher for cases infected with norovirus GII relative to cases in which norovirus GII was not identified. None of the other pathogens demonstrated a difference in severity between cases infected with that pathogen versus cases infected with other pathogens, and this result remained consistent with both severity scales. Children with gastroenteritis in the 6-12 month age range had significantly more severe mean gastroenteritis severity scores relative to children with gastroenteritis who belonged to the other age groups, according to both the Vesikari (12.7 v. 12.0, *P*=0.0002) and Clark (12.0 v. 11.4, *P*=0.0016) scales. There was not a statistically significant difference in severity of gastroenteritis based on other patient characteristics in either the Vesikari or Clark scoring systems.

Norovirus and sapovirus genogroup/genotype relationship with diarrhea. We successfully genotyped 282/337 (84%) of norovirus and 65/71 (92%) of sapovirus isolated from children with gastroenteritis; 31/32 (66%) of norovirus and 6/6 (100%) of sapovirus from asymptomatic hospital-based controls; and 51/74 (67%) of norovirus and 11/20 (55%) of sapovirus from asymptomatic community-based controls. The most frequently observed norovirus genotype was GII.4 (68%), followed by GII.6 (10%), and GII.17 (9%). Between the two norovirus genogroups, we found GII more frequently in gastroenteritis-associated infections than in asymptomatic controls (33% v. 9%, $P<0.01$). GI was found in 3% children with gastroenteritis and 4% of asymptomatic controls ($P=0.69$). Among those with genotype information, norovirus genotypes GI.1, GI.3, GI.5, GII.4, GII.6, GII.12, GII.14, GII.23 were significantly more frequent in children with gastroenteritis. We identified norovirus GII.8 and GII.13 only in asymptomatic controls.

Of the four genogroups of sapovirus, we most frequently observed GI (49%), followed by GII (30%), GIV (11%), and GIV (10%). Among those with genotype information, 65 were gastroenteritis-associated, and 16 were associated with asymptomatic controls from the community or hospital settings. Among the four genogroups, we found GI, GII, GIV, and GV more frequently in children with gastroenteritis (4%, 2%, 1%, 1%, respectively) than in asymptomatic children (1%, 0.5%, 0.4%, 0.2%, respectively). When analyzed by genotype, 12 of the 19 known genotypes were identified. Within these 12 genotypes, we most frequently identified GI/2 (32%), followed by GI/1 (15%), GII/4 (17%), GIV/1 (9%) and GV/1

(9%). Genotypes GI/1, GI/2, GI/3, GI/5, GII/1, GII/2, GII/4, GII/5, GII/8, GIV/1, and GV/1 were more frequent in children with gastroenteritis than asymptomatic controls. Within the 12 genotypes identified, we only detected genotype GI/6 more frequently in asymptomatic controls compared with gastroenteritis cases, but this difference was not statistically significant.

Rotavirus genotype relation to diarrhea, vaccination status, and disease severity. Rotavirus infections peaked from July through December, which correspond with the colder months in Lima (Figure 5). Of the 127 rotavirus-positive samples (98 cases, 28 controls), 81 (64%) had typable G and P types. Among cases with typable rotavirus, we most frequently identified the heterotypic strain G12P[8] (54/81, 67%). Of the 54 children with G12P[8]-associated gastroenteritis, 48 (89%) had received Rotarix™ dose one, and 45 (83%) had received Rotarix™ dose two. Among the 9 children (2 cases, 7 controls) infected with G1P[8], the vaccine strain of rotavirus, 8 (2 cases, 6 controls) had received one dose, and 4 (2 cases, 2 controls) had received two doses of Rotarix™. Having received one dose of Rotarix™ did not significantly impact the severity of diarrhea among children with rotavirus-associated gastroenteritis compared with their unimmunized children (median Vesikari score 12.5 v. 13 [both moderate]; median Clark score 11.5 v. 12.5 [both severe], respectively), although both scales revealed a 0.5 point trend toward increased severity among unimmunized children. Having received two doses of Rotarix™ did not significantly impact the severity of diarrhea among children with rotavirus-associated gastroenteritis compared with their unimmunized children

(median Vesikari score 12 v. 13 [both "moderate"]; median Clark score 11 v. 12 [both "severe"], respectively), although both scales revealed a one point trend toward increased severity among unimmunized children.

DISCUSSION

Using a comprehensive panel of microbiological assays, we performed this hospital-based case-control study to better characterize the etiology of medically-attended gastroenteritis in a developing setting following the implementation of a universal vaccine against rotavirus. By including asymptomatic control children without gastroenteritis, we derived burden estimates adjusted for the occurrence of asymptomatic enteropathogens often seen in children living in fecally contaminated environments, and derived an AF for every pathogen that was independently associated with medically-attended gastroenteritis in regression models, adjusting for interactions and confounding effects of coinfecting enteropathogens. These pathogen-specific adjusted AFs estimate the proportion of medically-attended gastroenteritis that could be prevented with targeted interventions such as effective vaccines. We further described the genetic epidemiology of the three most frequently identified viral pathogens, norovirus, rotavirus, and sapovirus, in order to inform these control measures.

This study revealed several noteworthy findings about young children with medically-attended gastroenteritis. First, despite our detection of a wide variety of viruses, bacteria, and parasites in the stools of young children in this setting, a small

number contributed to the majority of gastroenteritis cases, most notably norovirus GII, rotavirus, sapovirus, and astrovirus. The combined burden of gastroenteritis attributable to these four viruses is 53.4%. The only bacteria independently associated with gastroenteritis in this population, *Shigella* and St+ producing ETEC, had a combined attributable fraction of only 5.3%. Other organisms, such as *Campylobacter* spp., *Cryptosporidium*, *Aeromonas,* and *Vibrio cholerae* O1, which have been associated with moderate-to-severe diarrhea in multi-center studies conducted in Africa and Asia (Kotloff et al. 2013), were remarkably absent (in the case of cholera) or not associated with gastroenteritis in this hospital-based South American population. These differences in circulating pathogens could be due to population differences in nutritional status or medical comorbidities, in addition to differences in the study design, definition gastroenteritis, and measures of disease severity (Ballard, Saito, et al. 2015b). Neither our study nor the Global Enterics Multicenter Study (GEMS) found an association between EPEC and acute gastroenteritis, despite the known causal relationship between typical EPEC infection and diarrhea (M M Levine et al. 1978). However, we did not conduct studies to determine whether our EPEC isolates represented typical or atypical strains, which demonstrated different virulence characteristics (Trabulsi, Keller, and Gomes 2002). Interestingly, co-infection with any parasite was associated with a decreased risk of gastroenteritis. Similar to adults from endemic settings in whom *Trichuris* infection appears protective for gastroenteritis (Ballard, Reaves, et al. 2015b), we speculate that parasitic infection in our population serves as a proxy for frequent previous infection and consequent enhanced immunity to enteric

pathogens. Alternately, this could represent a measurement bias as a result of dilution of stool and the subsequent decreased parasite detection sensitivity could occur during watery diarrheal episodes.

Defining gastroenteritis disease severity is notoriously difficult, and results in the inability to accurately compare results across studies. Here, we prospectively collected information about the clinical features of disease in children presenting with gastroenteritis. We demonstrated large discrepancies between the modified 20-point Vesikari and 24-point Clark scale in regard to the definition of severe diarrhea. Despite these differences, both scales demonstrated a significantly higher gastroenteritis severity in children with norovirus GII positive v. GII negative gastroenteritis episodes, and episodes in children aged 6-12 months versus other ages. The primary concern related to discrepancies in definitions of severe gastroenteritis is with regard to their use in disease burden estimates and intervention efficacy studies. The GEMS study, which was the pivotal study evaluating diarrhea disease burdens across Africa and Asia, employed different definitions of both diarrhea and "moderate-to-severe" disease. As discussed elsewhere (Ballard, Saito, et al. 2015b), the reliance of this study on the WHO definition of diarrhea, and its failure to include children with vomiting-only gastroenteritis episodes, likely biased the study toward a lower norovirus-attributable fraction of disease. Likewise, GEMS did not prospectively evaluate diarrhea severity or use an accepted severity scoring system, such as the Vesikari or Clark scales. Rather, it evaluated severity at a single point during the episode and

categorized it as “moderate-to-severe” if the child had at least one of the following at the time of evaluation: sunken eyes, loss of skin turgor, visible blood in loose stools, utilization of intravenous hydration, or hospital admission for diarrhea or dysentery (Kotloff et al. 2013). The dysentery-focus of these severity criteria increases the likelihood of enrolling cases with bacterial rather than viral gastroenteritis etiologies.

Following the implementation of pediatric vaccines against rotavirus in Peru, norovirus GII has emerged as the enteropathogen with the highest AF (29%) of all pathogens identified in this study. Relative to children with non-norovirus-associated gastroenteritis, children with norovirus-associated gastroenteritis demonstrated a higher mean severity score on both the Vesikari and Clark severity scales. Individuals with norovirus GII-associated gastroenteritis consistently demonstrate more severe clinical features relative to individuals with norovirus GI-associated gastroenteritis (Ballard, Reaves, et al. 2015a). However, there are increasing reports of norovirus causing severe disease, especially in immunologically naïve individuals, such as travelers to Cusco, Peru, 91% of whom were confined to bed during norovirus-associated gastroenteritis episodes (Jennings, Tilley, Ballard, Villanueva, Costa, Lopez, Steinberg, Luna, et al. 2017). Our finding that 6-12 month old infants experience more severe disease serves supports this, since maternal antibodies have waned by this age and weaning increases the risk of diarrhea among young children (Santos et al. 2015). In order to prevent severe disease, vaccines against norovirus must target circulating norovirus

genotypes and variants (Saito et al. 2014). This is challenging because circulating strains of norovirus are genetically diverse, as demonstrated by our identification of 12 different genotypes among hospital cases. Nevertheless, the GII.4 genotype predominated overwhelmingly, followed by temporally clustered GII.17 and GII.6 cases, suggesting that, similar to the influenza vaccine model, surveillance systems could be useful in informing vaccine development.

In our hospital population with gastroenteritis, sapovirus was only slightly less genetically diverse compared with community setting, where all four human sapovirus genotypes and 14 of the 19 known genotypes affecting humans were recently identified (G. J. Sanchez et al. 2017). Among our hospital cases, the genetic diversity (consisting of all four genogroups and 12 genotypes) of sapovirus is more diverse when compared to other developing and developed hospital settings. Only sapovirus GI was detected in Vietnam and Bangladesh (Hansman et al. 2004; Shuvra Kanti Dey et al. 2007), and only sapovirus GI and GII were reported in a U.S. study (Chhabra et al. 2013). In Japan (S K Dey et al. 2012) and the Philippines (X. Liu et al. 2015), sapovirus GV and GIV, respectively, were not reported. We detected sapovirus GI more frequently than GII (50% v. 31%), similar to the findings of a community-based study in Nicaragua (Bucardo et al. 2014). For unknown reasons, sapovirus GI may be more pathogenic, as it is frequently detected in diarrhea episodes and with lower Cq values than the other genotypes (G. J. Sanchez et al. 2017).

The diversity of circulating rotavirus strains in Peru has not been well characterized since the 2009 introduction of vaccines rotavirus to the childhood immunization schedule in Peru. In this population of young children exposed to the universal Rotarix™ vaccination program, we noted a predominance of G12P[8] in this population. Since 1998, the G12 genotype has been isolated in Asia, Europe, and the Americas, suggesting global expansion and propagation of this strain (Rahman et al. 2007; Soares et al. 2012). G12 has been previously reported in Latin American countries, including Brazil and Argentina, associated with P[6], and P[9], (Rahman et al. 2007; Pietruchinski et al. 2006; Castello et al. 2009; Stupka et al. 2012; Soares et al. 2012). In one Spanish study, G12 was most commonly associated with P[8], similar to our findings (Cilla et al. 2010). A recent outbreak of G12P[8]-associated gastroenteritis among adults in the United States provides additional evidence of the pathogenic importance of this emergent strain (Pacilli et al. 2015). The Rotarix™ vaccine used throughout Latin America and the developing world is not composed of a G12 reassortant rotavirus, and vaccine efficacy against this strain is not yet known. However, RotaTeq™ appears to have an approximate efficacy of 80% against G12 (D. C. Payne et al. 2013). This suggests that the use of RotaTeq™ and similar vaccines with G12 coverage may be necessary to control rotavirus in the future.

Limitations of the study include the endemicity of many enteropathogens in the study population, creating and environment in which the same organism are detected in cases and controls, which could result in an underestimation of the

contribution of specific pathogens to the burden of medically-attended gastroenteritis. Calicivirus genotyping limitations include the inability to genotype all of the norovirus and sapovirus speciments. In addition, norovirus and sapovirus genotyping was based on partial sequencing of the VP2 and VP1 genes, respectively, and the polymerase genes were not analyzed. Therefore, we did not evaluate recombinant strains or genotypic variants.

CONCLUSION

In summary, this hospital-based case-control study documents the substantial contribution of caliciviruses—particularly norovirus and sapovirus—to medically-attended pediatric gastroenteritis in a developing setting following Rotarix™ vaccine implementation. These findings, in addition to studies of diarrhea in the community setting, strongly suggest the need for a triple vaccine targeting rotavirus, norovirus, and sapovirus, to prevent diarrhea, malnutrition, and hospitalization among young children.

V. CONCLUSIONS

In conclusion, these studies helps extend the knowledge of the complex epidemiology of norovirus infection and disease in two developing country populations, young children and the Peruvian military. The hospital-based case-control study of young children with and without diarrhea demonstrates the substantial contribution of caliciviruses—particularly norovirus and sapovirus—to medically-attended pediatric gastroenteritis in a developing setting following Rotarix™ vaccine implementation. These findings srongly suggest the need for a triple vaccine targeting rotavirus, norovirus, and sapovirus to prevent diarrhea, malnutrition, and hospitalization among young children in developing settings. In military adults stationed in the Peruvian Amazon Basin, norovirus also contributed significantly to disease. In this setting, norovirus is second only to *Shigella* as the leading cause of gastroenteritis. Analysis of the cost-effectiveness of norovirus vaccine implementation in Peruvian military service members demonstrates that military gastroenteritis vaccine acquisition strategies should focus on vaccines against *Shigella* and norovirus.

In summary, molecular diagnostics have drastically improved our understanding of gastrointestinal pathogens. Investigations of the etiology of gastroenteritis in developing settings suggest that the disease burden of caliciviruses, specifically norovirus and sapovirus, are high, making it a leading cause of diarrhea-associated morbidity and mortality worldwide. In high-income countries, norovirus infection frequently affects children, the elderly, immunocompromised individuals, health

care workers, food handlers, travelers, and the military. In developing settings, young children experience the highest incidence of norovirus-associated disease; however, it also affects adults in special populations, such as military personnel, in these settings. Immunity to norovirus is variant- or genotype-specific, and has limited duration. .

Vaccines against norovirus are rapidly approaching the market. These vaccines are based on virus like particle or P particle subunit production in expression systems. One of the vaccine implementation challenges is that many distinct population groups are affected. However, disease burden data, particularly in developing setting populations, are scarce. Further, remaining methodological controversies over norovirus disease estimation remain. Due to the high sensitivity of molecular detection methods, norovirus is frequently identified in stools from healthy individuals, making the interpretation of disease attribution complex. Critical knowledge gaps also include understanding natural immunity to norovirus and correlates of protection, the roles of specific age groups in norovirus transmission, and the implications of human genetic susceptibility to norovirus for population health, viral evolution, and vaccines.

VI. TABLES

Table 1. Pathogens Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin

Table 2. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Peruvian Military Personnel in the Amazon Basin

Table 3. Norovirus Genotypes/Variants Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin

Table 4. Pathogens Detected in Peruvian Military Personnel in the Amazon Basin with Norovirus-positive versus Norovirus-negative Stool Samples

Table 5. Clinical Features of GI- versus GII-associated Norovirus Gastroenteritis in Peruvian Military Personnel in the Amazon Basin

Table 6. Parameter Estimates Used for the Pathogen-Specific Gastroenteritis Vaccine Cost Effectiveness Analysis Applied to the Peruvian Military Population

Table 7. Baseline Estimates of Total Costs, Effectiveness Measures, and Cost-Effectiveness Ratios for Pathogen-Specific Analysis of Vaccines Against *Campylobacter,* ETEC, *Shigella,* and Norovirus in a Peruvian Military Population in the Amazon Basin

Table 8. Population Characteristics and Pathogens Detected in Stool Samples from Children Younger than Five Years of Age with and without Medically-attended Gastroenteritis in Peru

Table 9. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Children in Lima, Peru

Table 10. Clinical Features and Severity of Gastroenteritis by Pathogen in Children Younger than Five Years of Age in Peru

Table 1. Pathogens Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin

Organisms	**Cases** Number (%)	**Controls** Number (%)	***P*-value**
Viruses	(n=184)	(n=176)	
Norovirus*	26 (14.1)	14 (8.0)	0.067
GI	7 (3.8)	5 (2.8)	0.771
GII	20 (10.9)	9 (5.1)	0.053
Bacteria	(n=200)	(n=198)	
Shigella	**43 (21.5)**	**8 (4.0)**	**<0.001**
ETEC†	**16 (8.1)**	**2 (1.0)**	**0.001**
Campylobacter	1 (0.5)	4 (2.0)	0.214
Salmonella	3 (1.5)	0 (0.0)	0.248
Plesiomonas	2 (1.0)	1 (0.0)	0.999
Vibrio	1 (0.5)	0 (0.0)	0.999
Yersinia	0 (0.0)	0 (0.0)	0.999
Parasites	(n=196)	(n=194)	
Trichuris	**30 (15.3)**	**52 (26.8)**	**0.006**
Ascaris	88 (45.4)	71 (36.2)	0.080
Giardia	42 (21.4)	38 (20.0)	0.707
Uncinaria	27 (13.5)	30 (20.1)	0.669
Ancylostoma	7 (3.6)	3 (1.6)	0.337
Strongyloides	2 (1.0)	7 (3.6)	0.104

Abbreviations: GI = norovirus genogroup I, GII = norovirus genogroup II, No. = number, ETEC = Enterotoxigenic *Escherichia coli*

*One participant with diarrhea tested positive for both norovirus GI and GII infection

†RT-PCR for Enterotoxigenic *Escherichia coli* was not performed on 6 samples

Table 2. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Peruvian Military Personnel in the Amazon Basin

	Crude			Adjusted[a]		
Risk Factor	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
NV GII	2.4	(1.1–5.6)	0.03	3.4	(1.3–8.7)	0.012
Shigella	6.5	(3.0–14.2)	<0.001	6.7	(3.0–14.8)	<0.001
Enterotoxigenic *Escherichia coli*	8.5	(1.9–37.6)	0.005	9.1	(2.0–41.2)	0.004
Trichuris	0.5	(0.3–0.8)	0.006	0.4	(0.3–0.8)	0.005

Abbreviations: NV = norovirus, GII = Norovirus genogroup II, OR = Odds Ratio, CI = Confidence Interval

[a] Adjusted model includes Norovirus GII, *Shigella*, Enterotoxigenic *Escherichia coli*, and *Trichuris*

Table 3. Norovirus Genotypes/Variants Detected in Stool Samples from Peruvian Military Personnel with and without Gastroenteritis in the Amazon Basin

Genotype/Variant (N=25)	**Total** Number (%) [a]	**Cases** Number (%)	**Controls** Number (%)
Genogroup I			
GI.1	1 (4)	1 (4)	0 (0)
GI.3	1 (4)	1 (4)	0 (0)
GI.4	7 (28)	4 (16)	3 (12)
GI.5	1 (4)	0 (0)	1 (4)
GI.7	1 (4)	1 (4)	0 (0)
Genogroup II			
GII.4/2006b Den Haag	6 (24)	6 (24)	0 (0)
GII.5	1 (4)	1 (4)	0 (0)
GII.6	1 (4)	1 (4)	0 (0)
GII.14	1 (4)	1 (4)	0 (0)
GII.15	1 (4)	1 (4)	0 (0)
GII.16	2 (8)	2 (8)	0 (0)
GII.17	2 (8)	2 (8)	0 (0)
Total	25 (100)	21 (84)	4 (16)

Abbreviations: GI = norovirus genogroup I, GII = norovirus genogroup II

[a] In addition to those reported in the table, 16 noroviruses were untypeable because no amplicon was produced on conventional PCR (0 norovirus GI and 7 GII) or because there was no match for the sequence in GenBank (1 norovirus GI and 8 GII)

Table 4. Pathogens Detected in Peruvian Military Personnel in the Amazon Basin with Norovirus-positive versus Norovirus-negative Stool Samples

	Diarrhea Cases		
	Norovirus Negative (n=158) Number (%)	**Norovirus Positive (n=26)** Number (%)	***P*-Value**
Bacterial Pathogens	(n=158)	(n=26)	
Shigella	36 (22.8)	4 (15.4)	0.608
Enterotoxigenic *Escherichia coli*	12 (7.7)	4 (15.4)	0.253
Salmonella	3 (1.9)	0 (0.0)	0.999
Plesiomonas	2 (1.3)	0 (0.0)	0.999
Campylobacter	1 (0.6)	0 (0.0)	0.999
Vibrio	1 (0.6)	0 (0.0)	0.999
Yersinia	0 (0.0)	0 (0.0)	0.999
Parasites	(n=155)	(n=26)	
Trichuris	19 (12.3)	7 (26.9)	0.067
Ascaris	57 (36.8)	6 (23.1)	0.191
Giardia	33 (21.3)	7 (26.9)	0.609
Uncinaria	23 (14.8)	2 (7.7)	0.539
Ancylostoma	6 (3.9)	0 (0.0)	0.596
Strongyloides	2 (1.3)	0 (0.0)	0.999

[a] Number of missing results were as follows: *Shigella*-0; ETEC-2; *Salmonella*-0; *Plesiomonas*-0; *Campylobacter*-0; *Vibrio*-0; *Yersinia*-0; *Trichuris*-3; *Ascaris*-3; *Giardia*-3; *Uncinaria*-0; *Ancylostoma*-3; *Strongyloides*-3

Table 5. Clinical Features of GI- versus GII-associated Norovirus Gastroenteritis in Peruvian Military Personnel in the Amazon Basin

Clinical Feature	**NV GI** **(n=6)** [a] Number (%)	**NV GII** **(n=19)** [a] Number (%)	***P*-Value**
Abdominal pain	**3 (50.0)**	**18 (94.7)**	**0.031**
Tenesmus	3 (50.0)	14 (73.6)	0.344
Abdominal cramps	3 (50.0)	13 (68.4)	0.630
Nausea	1 (16.7)	5 (26.3)	0.999
Vomiting	0 (0.0)	3 (15.8)	0.554
Hematochezia	0 (0.0)	1 (5.3)	0.999
Fever	0 (0.0)	0 (0.0)	0.999

Abbreviations: GI = Norovirus genogroup I, GII = Norovirus genogroup II, No. = number, SD = standard deviation

[a] The one participant co-infected with Norovirus GI and GII was excluded from this analysis

Table 6. Parameter Estimates Used for the Pathogen-Specific Gastroenteritis Vaccine Cost Effectiveness Analysis Applied to the Peruvian Military Population

		Estimate	Reference
Deployment parameters			
	Yearly deployment size	120,660	("Global Fire Power" 2016)
	Deployment duration in months	3.5	Unpublished data (Guerra R)
	Time-horizon (years)	1	(Tallant et al. 2014b)
	Discount rate	0.00%	(Tallant et al. 2014b)
Gastroenteritis			
	Monthly incidence (as percent)	6.5%	(Ballard, Reaves, et al. 2015a)
	Vaccine covered pathogen prevalence	14.1%	(Ballard, Reaves, et al. 2015a)
Vaccine acquisition program			
	Vaccine coverage	75%	(Riddle et al. 2008)
	Vaccine efficacy	80%	(Riddle and Tribble 2008)
	[P] of adverse event needing treatment	0.0125%	(Riddle et al. 2008)
	Cost of vaccine administration	$0.50	(A. D. Clark et al. 2009)
	Cost per dose of vaccine	$7.50	(A. D. Clark et al. 2009)
	Number of doses needed	2	(Mirelman et al. 2015a; Tallant et al. 2014b)
	Cost per adverse event treated	$104	Unpublished data (Guerra R)
Current management approach			
	[P] seeking medical treatment for illness	76.90%	(Ballard, Reaves, et al. 2015a)
	[P] not seeking medical treatment	23.10%	(Ballard, Reaves, et al. 2015a)
	[P] medical evacuation (MEDEVAC)	0.0329%	(Tallant et al. 2014b)
Treatment type provided			
	[P] ambulatory (outpatient)	63.50%	(Ballard, Reaves, et al. 2015a)
	[P] confinement to bed rest	32.00%	(Ballard, Reaves, et al. 2015a)
	[P] hospitalization	4.50%	(Ballard, Reaves, et al. 2015a)
Cost of treatment type provided ($)			
	Medical evacuation (note cost is per hour)	S/.10,007.76	Unpublished data (Guerra R)
	Hospitalization (deployed)	S/.473.36	Unpublished data (Guerra R)
	Sick in quarters/Confinement to bed rest	S/.79.84	Conservative estimate based on (Tallant et al. 2014b)
	Ambulatory	S/.79.84	Unpublished data (Guerra R)
	Annual investment on treatment trials	n/a	n/a

Abbreviations: [P] = probability

Table 7. Baseline Estimates of Total Costs, Effectiveness Measures, and Cost-Effectiveness Ratios for Pathogen-Specific Analysis of Vaccines Against *Campylobacter,* ETEC, *Shigella,* and Norovirus in a Peruvian Military Population in the Amazon Basin

Pathogen	***Campylobacter***	**ETEC**	***Shigella***	**Norovirus**
Annual vaccine-preventable illness events	137	2,223	5,902	3,870
Annual cost of immunizing target population	$1,447,920	$1,447,920	$1,447,920	$1,447,920
Annual number of gastroenteritis events (pre-licensure: post-licensure)				
Outpatient	66:10	1,082:162	2,872:430	1,884:282
Sick in quarters/Confinement to bed rest	34:5	550:82	1459:219	957:144
Hospitalization	5:1	77:12	205:31	135:20
Aeromedical evacuation	0:0	1:0	2:0	1:0
Total cost of care for vaccine acquisition strategy	$543	$8,802	$23,364	$15,322
Total cost of care for current management	$3,622	$58,681	$155,758	$102,148
Annualized cost of care averted by vaccine acquisition strategy	$3,079	$49,879	$132,394	$86,826
Total duty days lost vaccine acquisition strategy	23	240	940	368
Total duty days lost current management	116	1,201	4,669	1,839
Annualized duty days lost averted due to vaccine acquisition strategy	93	961	3,759	1,471
Cost effectiveness ratio (US$/duty days lost averted)	$15,598	$1,456	$350	$926
Cost effectiveness ratio (US$/diarrhea day averted)	$2,418	$246	$60	$229

Abbreviations: ETEC = Enterotoxigenic *Escherichia coli*

Table 8. Population Characteristics and Pathogens Detected in Stool Samples from Children Younger than Five Years of Age with and without Medically-attended Gastroenteritis in Peru

Population characteristics, n=1,788	**CASES, n=932**		**CONTROLS, n=856**		***P*-value**
Male, number (%)	528	56.7%	434	50.7%	0.01
Age (mos), mean (SD)	21.5	13.0	24.9	16.0	<0.01
0-5 mos, number (%)	19	2.0%	24	2.8%	0.11
6-11 mos, number (%)	191	20.5%	155	18.1%	<0.01
12-23 mos, number (%)	410	44.0%	260	30.4%	<0.01
24-35 mos, number (%)	171	18.3%	146	17.1%	0.26
36-47 mos, number (%)	79	8.5%	152	17.8%	<0.01
48-59 mos, number (%)	62	6.7%	119	13.9%	<0.01
Socieoeconomic score , median (range)	39	(4-59)	32	(6-54)	<0.01
Improved sanitation (WHO definition), number (%)	872	93.6%	339	39.6%	<0.01
Improved water source (WHO definition), number (%)	839	90.2%	353	41.2%	<0.01
Hematocrit, mean (SD)	35.0	2.8	35.5	2.6	<0.01
Blood group					
O, number (%)	578	81.4%	673	82.1%	0.39
A, number (%)	92	13.0%	106	12.9%	0.52
B, number (%)	37	5.2%	37	4.5%	0.3
AB, number (%)	3	0.4%	4	0.5%	0.58
Rhesus factor positive, number (%)	704	99.2%	817	99.6%	0.32
Weght for age Z-score, mean (SD)	0.11	1.28	0.53	1.23	<0.01
Weight for length/height Z-score, mean (SD)	0.18	1.74	0.57	1.42	<0.01
Length/height for age Z-score, mean (SD)	0.06	2.28	0.31	1.81	0.02
BMI for age Z-score, mean (SD)	0.15	1.92	0.52	1.55	<0.01
Rotavirus dose 1, number (%)	876	96.6%	676	92.0%	<0.01
Rotavirus dose 2, number (%)	833	91.8%	628	85.4%	<0.01
Season					
Summer (December - February), number (%)	247	26.5%	154	18.0%	<0.01
Fall (March - May), number (%)	298	32.0%	255	29.8%	<0.01
Winter (June - August), number (%)	172	18.5%	212	24.8%	0.11
Spring (September - November), number (%)	215	23.1%	235	27.5%	<0.01
Microbes, number (%)	677	72.6%	378	44.2%	<0.01
Viruses	528	56.7%	178	20.8%	<0.01
Aichivirus	1	0.2%	1	0.3%	1
Astrovirus	48	5.2%	23	2.7%	<0.01
Norovirus	337	36.2%	106	12.4%	<0.01
GI	29	3.1%	30	3.5%	0.69
GII	312	33.5%	77	9.0%	<0.01
Rotavirus	98	10.5%	29	3.4%	<0.01
Sapovirus	81	8.7%	26	3.0%	<0.01

Bacteria	361	38.7%	210	26.5%	<0.01
Aeromonas	27	2.9%	15	1.9%	0.21
Campylobacter	32	3.4%	14	1.8%	0.04
Campylobacter coli	2	0.2%	3	0.4%	0.66
Campylobacter jejuni	30	3.2%	11	1.4%	0.02
E. coli	297	31.9%	179	22.6%	<0.01
DAEC	154	16.5%	105	13.2%	0.06
EAEC	66	7.1%	29	3.7%	<0.01
EPEC	48	5.2%	35	4.4%	0.5
EIEC	2	0.2%	1	0.1%	1
ETEC	51	5.5%	22	2.8%	<0.01
ETEC (Lt+)	18	1.9%	13	1.6%	0.71
ETEC (St+)	27	2.9%	9	1.1%	0.01
ETEC (Lt+/St+)	6	0.6%	0	0.0%	0.03
STEC	12	1.3%	4	0.5%	0.13
STEC (STX1)	5	0.5%	0	0.0%	0.07
STEC (STX2)	3	0.3%	0	0.0%	0.25
STEC (STX1/STX2)	4	0.4%	4	0.5%	1
Plesiomonas	2	0.2%	0	0.0%	0.5
Salmonella	14	1.5%	14	1.8%	0.71
Shigella	21	2.3%	2	0.3%	<0.01
Shigella flexneri	8	0.9%	0	0.0%	<0.01
Shigella sonnei	10	1.1%	1	0.1%	0.01
Shigella other	3	0.3%	1	0.1%	0.375
Vibrio	0	0.0%	0	0.0%	1
Parasites	22	5.3%	103	15.1%	<0.01
Ascaris lumbricoides	2	0.5%	2	0.3%	0.64
Blastocystic hominis	1	0.2%	0	0.0%	0.38
Chilomastix mesnili	3	0.7%	9	1.3%	0.55
Cryptosporidium	4	1.0%	4	0.6%	0.49
Endolimax nana	0	0.0%	16	2.3%	<0.01
Entamoeba coli	3	0.7%	31	4.5%	<0.01
Giardia lamblia	13	3.1%	59	8.6%	<0.01
Trichuris trichura	0	0.0%	1	0.1%	0.99

Abbreviations: n = number, SD = standard deviation, mos. = months, BMI = body mass index, DAEC = diffuse-adhering *Escherichia (E.) coli,* EAEC = enteroaggregative *E. coli*, EPEC = enteropathogenic *E. coli*, EIEC = enteroinvasive *E. coli*, ETEC = enterotoxigenic *E. coli*, Lt+ = heat labile, St+ = heat stable, STEC = shigatoxigenic *E. coli,* STX1 = Shigatoxin 1, STX2 = Shigatoxin 2

Table 9. Results of the Logistic Regression Analysis Demonstrating the Relationship between the Presence of Specific Pathogens in Stool and Gastroenteritis Among Children in Lima, Peru

	Crude			Adjusted[a]		
Risk Factor	**OR**	**95% CI**	***P*-value**	**AOR**	**95% CI**	***P*-value**
Shigella	9.1	2.1-39.0	**0.003**	9.1	1.2-43.0	**0.005**
Norovirus GII	5.1	3.9-6.7	**<0.001**	6.4	4.4-9.4	**<0.001**
Rotavirus	3.4	2.2-5.1	**<0.001**	6.0	2.8-12.6	**<0.001**
Sapovirus	3.0	1.9-4.8	**<0.001**	3.6	2.0-6.6	**<0.001**
ETEC St+	3.5	1.6-7.2	**0.001**	3.1	1.2-8.2	**0.021**
Astrovirus	2.0	1.2-3.3	**0.009**	2.2	1.0-4.4	**0.005**
Parasite (Any)	0.3	0.2-0.5	**<0.001**	0.3	0.2-0.5	**<0.001**

Abbreviations: GII = Norovirus genogroup II, OR = Odds Ratio, CI = Confidence Interval,

[a] Adjusted model includes age, sex, socioeconomic status, *Shigella*, norovirus GII, rotavirus, enterotoxigenic *Escherichia coli* (St+ and Lt+/St+), sapovirus, astrovirus, and the presence of any parasite

Table 10. Clinical Features and Severity of Gastroenteritis by Pathogen in Children Younger than Five Years of Age in Peru

Clinical features (cases only)	All cases, n=932		Shigella, n=21		Norovirus GII, n=312		Rotavirus, n=98		ETEC St+/Lt+St+, n=33		Sapovirus, n=81		Astrovirus, n=48	
Vesikari score, mean (SD)	12.1	2.3	12.9	2.3	12.6	2.3	12.3	2.3	12.8	2.2	11.7	2.3	12.0	2.0
Mild, number (%)	231	25%	3	14%	52	17%	24	24%	6	18%	21	26%	12	0.25
Severe, number (%)	701	75%	18	86%	260	83%	74	76%	27	82%	60	74%	36	0.75
Clark score, mean (SD)	11.5	2.2	12.1	2.5	11.7	2.3	11.5	1.8	11.7	2.4	11.2	2.2	11.4	2.1
Mild, number (%)	84	9%	1	5%	26	8%	6	6%	4	12%	11	14%	4	8%
Moderate, number (%)	839	90%	20	95%	280	90%	92	94%	29	88%	70	86%	44	92%
Severe, number (%)	9	1%	0	0	6	2%	0	0	0	0	0	0	0	0
Diarrhea reported, number (%)	830	89%	21	100%	294	94%	92	94%	31	94%	76	94%	43	90%
Duration of diarrhea among those with diarrhea (days), mean (SD)	4.9	2.9	6.8	1.2	5.3	2.9	4.5	1.7	6.7	3.7	5	2.5	4.9	2.4
Maximum daily number of diarrheal stools, mean (SD)	4.7	2.9	6.0	3.0	5.0	2.6	4.4	3.1	4.7	3	4.5	2.3	4.3	2.5
Number of children with vomiting (%)	768	82%	15	71%	288	92%	86	88%	25	76%	69	85%	33	69%
Duration of vomiting among those with vomiting (days), mean (SD)	2.6	1.7	2.1	4.7	2.8	1.7	2.5	1.4	2.6	1.8	2.5	1.3	3.0	2.1
Maximum number of vomiting episodes in one day, mean (SD)	3.5	3.1	3.2	3.4	4.4	3.3	3.2	3.2	2.6	2.6	3.4	2.8	2.9	3.0
Fever reported, number (%)	511	55%	15	71%	176	56%	52	100 %	20	61%	27	33%	26	54%
Duration of fever among those with fever (days), mean (SD)	2.2	1.3	1.7	1	2.3	1.5	2	1.2	2.2	1.7	2.4	1.4	2.0	1.0
Behavioral symptoms irritable, number (%)	162	17%	1	5%	59	18%	9	9%	12	36%	13	16%	13	27%
Behavioral symptoms listless, number (%)	551	59%	16	76%	172	52%	65	66%	12	36%	47	58%	23	48%
Behavioral symptoms, seizure, number (%)	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Dehydration mild, number (%)	499	45%	14	67%	172	52%	48	49%	22	67%	49	60%	25	52%
Dehydration moderate to severe, number (%)	2	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Treatment given - oral rehydration, number (%)	930	100%	21	100%	312	100%	98	100 %	33	100 %	81	100 %	48	100%
Treatment given- intravenous (parenteral) rehydration, number (%)	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%

Abbreviations: GII = Genogroup II; ETEC = Enterotoxigenic Escherichia coli; St+ = Heat stabile toxin positive; Lt+/St+ Heat labile and heat stabile toxin positive; n = Number; SD = Standard deviation

VII. FIGURES

Figure 1. Map of Peru, Indicating the Peruvian Military Study Site of Iquitos in Peruvian Amazon Basin and Lima, the Capital City where the National Childre Hospital is Located

Figure 2. Flow Diagram of Peruvian Military Participant Enrollment and Sele of Participants for the Nested Case-Control Study

Figure 3. Economic model evaluating the cost-effectiveness of vaccines again specific gastroenteritis-associated pathogens for a Peruvian military populatio taken from (Tallant et al. 2014a)

Figure 4. One-way Sensitivity Analyses for the Cost-Effectiveness Analyses of Vaccines Against (A) *Campylobacter*; (B) ETEC; (C) *Shigella*; and (D) Norovirus Peruvian Military Population in the Amazon Basin

Figure 5. Rotavirus prevalence over study period demonstrating the relation between rotavirus seasonality (red line) and rotavirus genotype G12P[8] prev (green line), Peru, 2013-15

Figure 1. Map of Peru, Indicating the Peruvian Military Study Site of Iquitos in the Peruvian Amazon Basin and Lima, the Capital City where the National Children's Hospital is Located

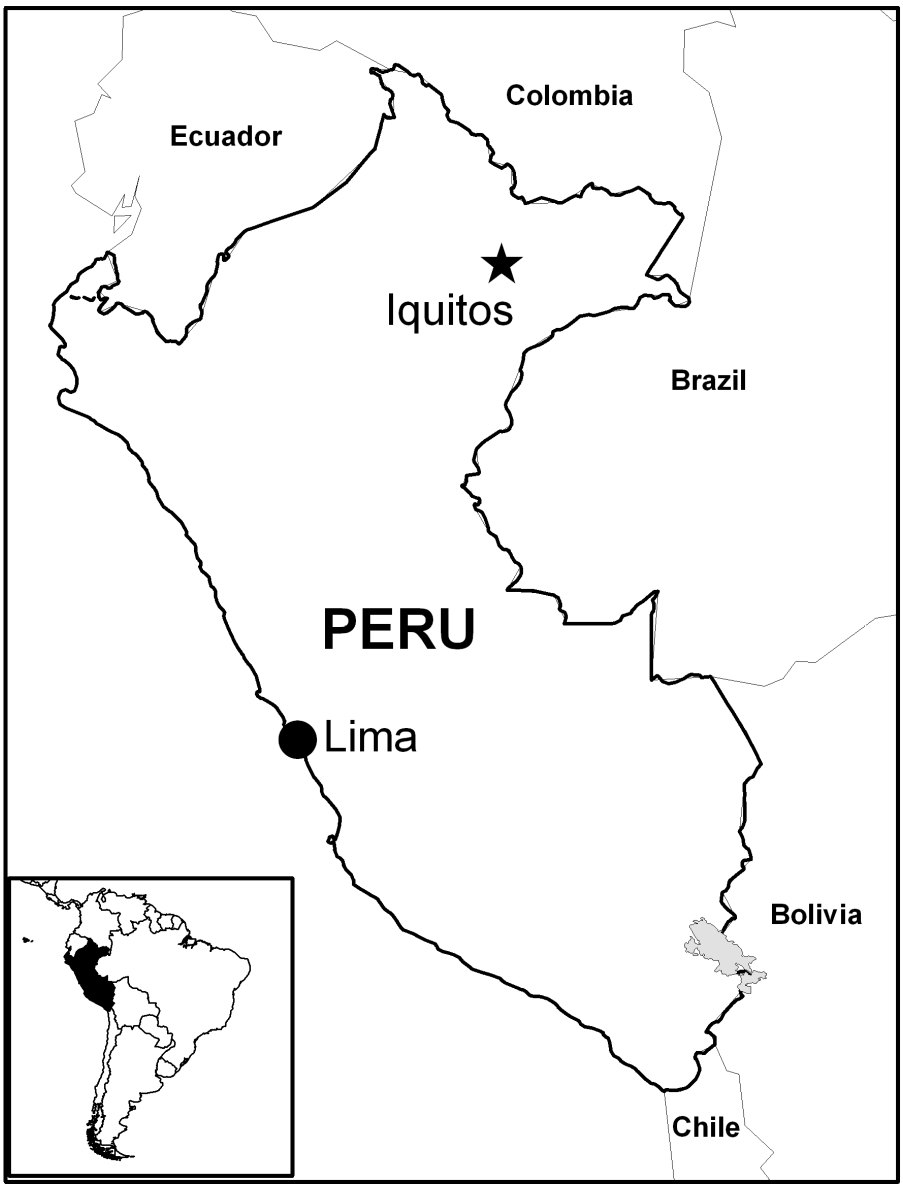
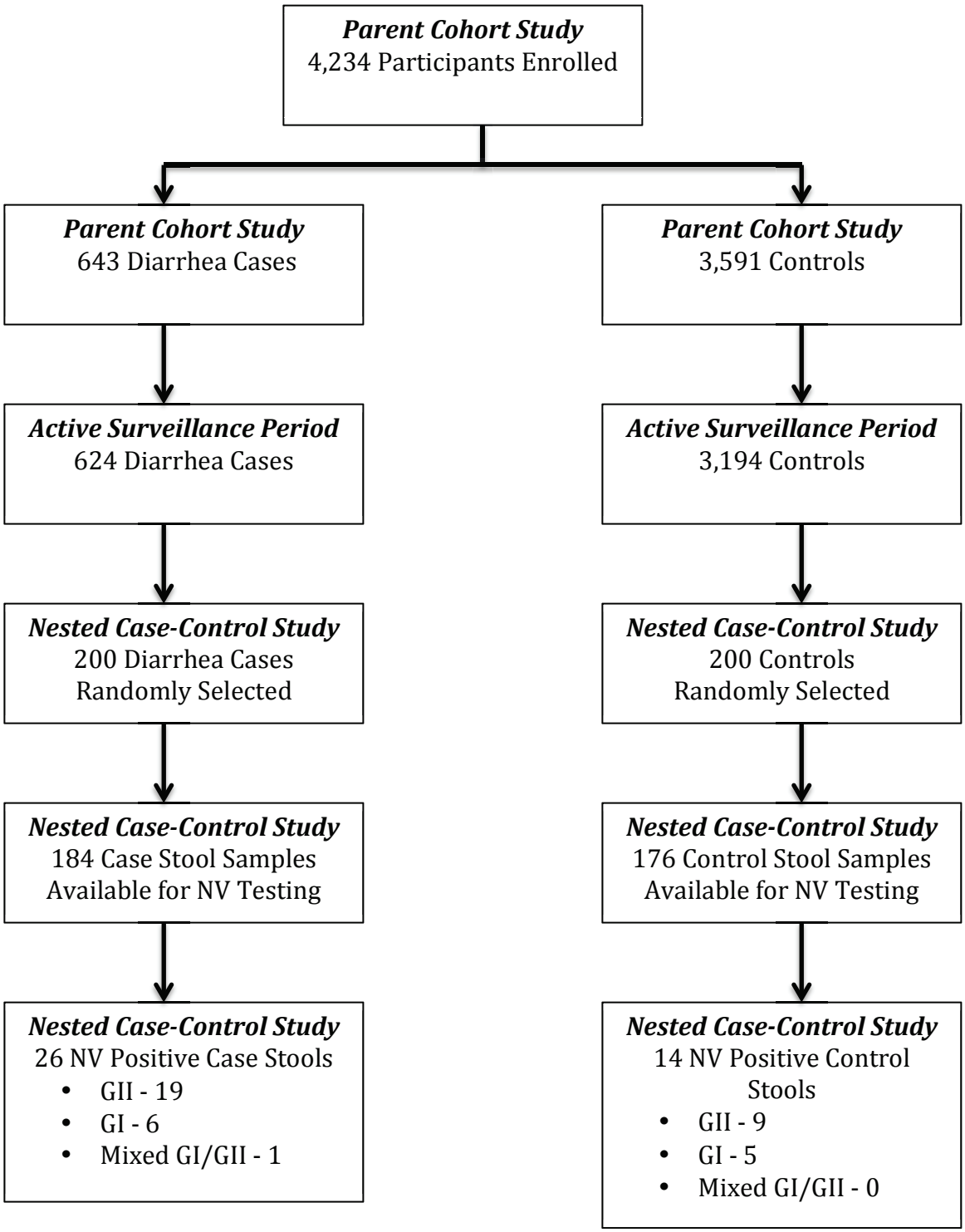


Figure 2. Flow Diagram of Peruvian Military Participant Enrollment and Selection of Participants for the Nested Case-Control Study



Abbreviations: NV = norovirus, GI = Norovirus genogroup I, GII = Norovirus genogroup II

Figure 3. Economic model evaluating the cost-effectiveness of vaccines against specific gastroenteritis-associated pathogens for a Peruvian military population, taken from (Tallant et al. 2014a)

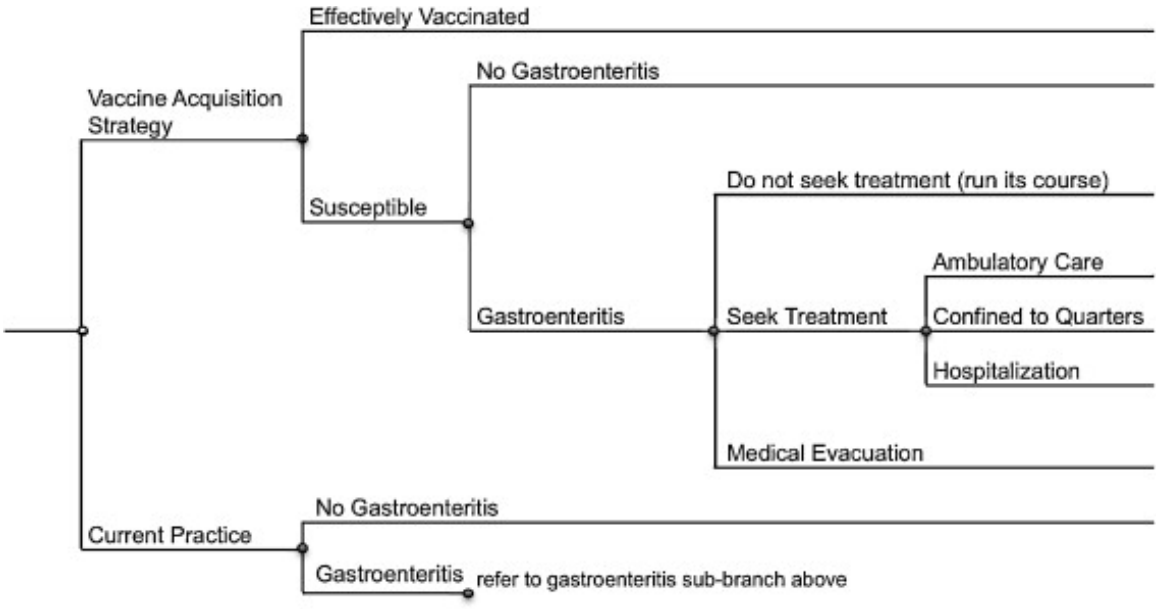
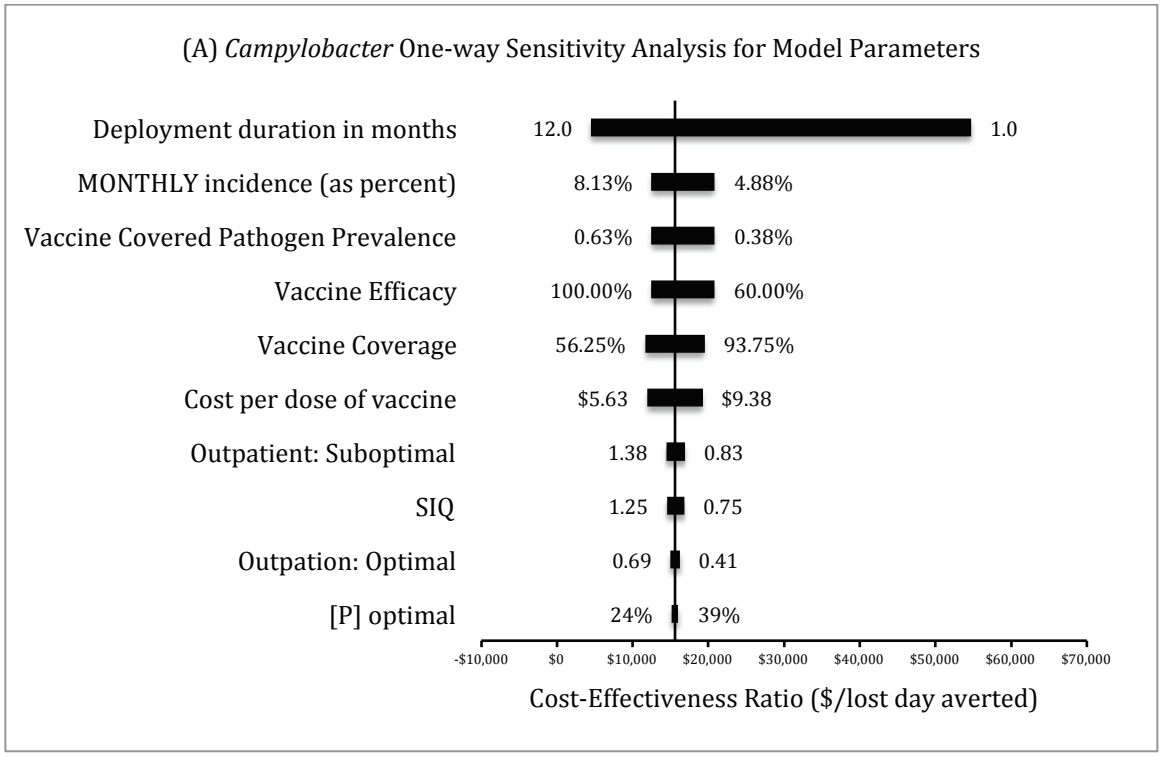
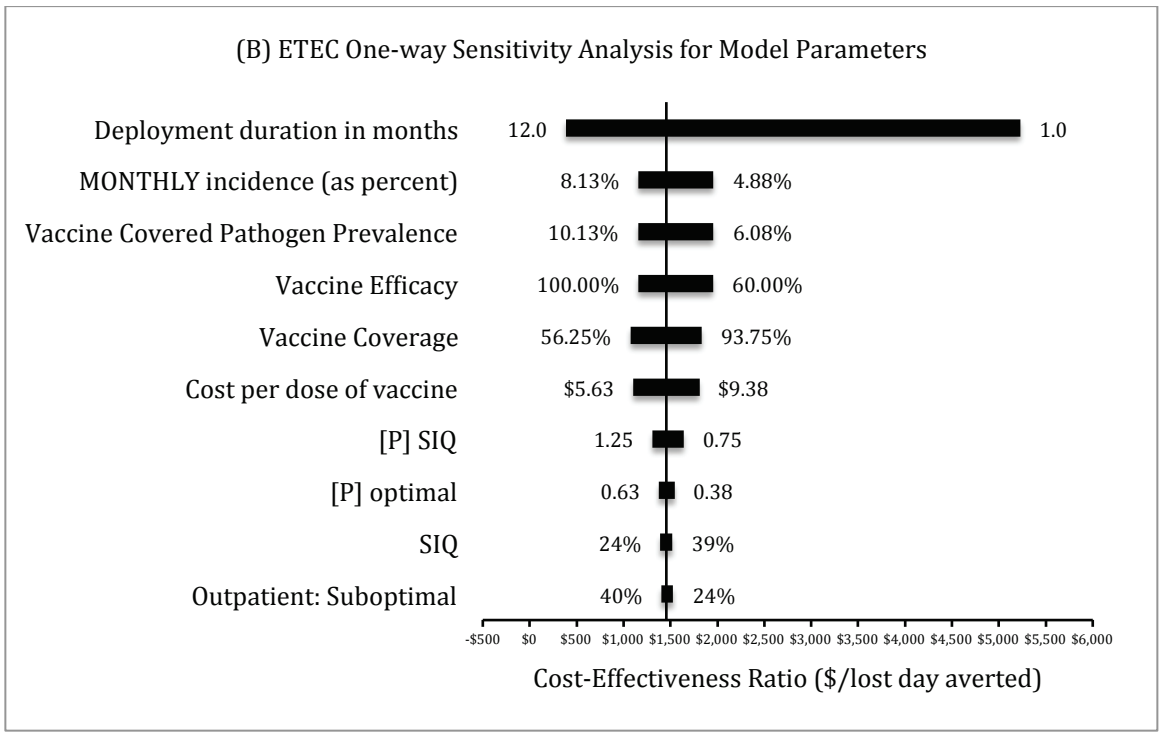
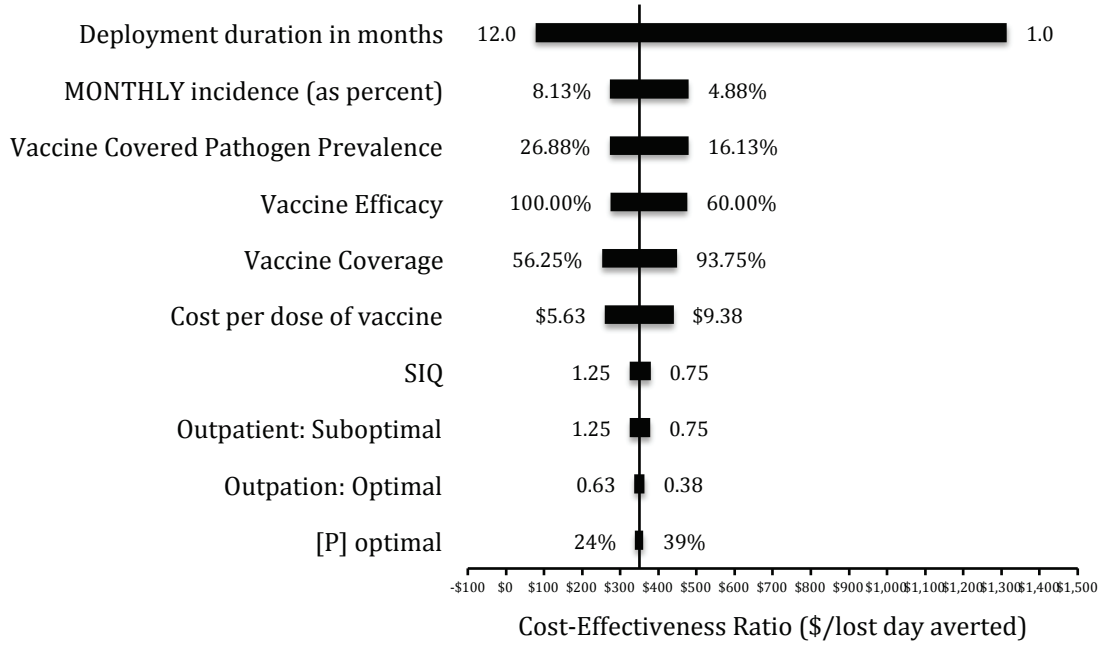


Figure 4. One-way Sensitivity Analyses for the Cost-Effectiveness Analyses of Vaccines Against (A) *Campylobacter*; (B) ETEC; (C) *Shigella*; and (D) Norovirus in the Peruvian Military Population in the Amazon Basin





(C) *Shigella* One-way Sensitivity Analysis for Model Parameters



(D) Norovirus One-way Sensitivity Analysis for Model Parameters

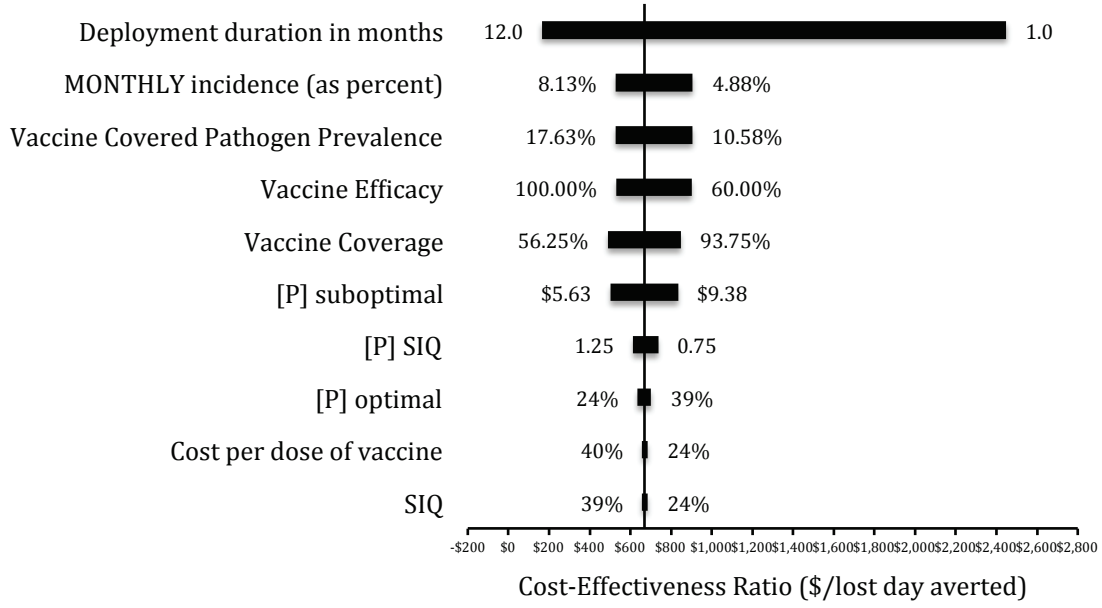
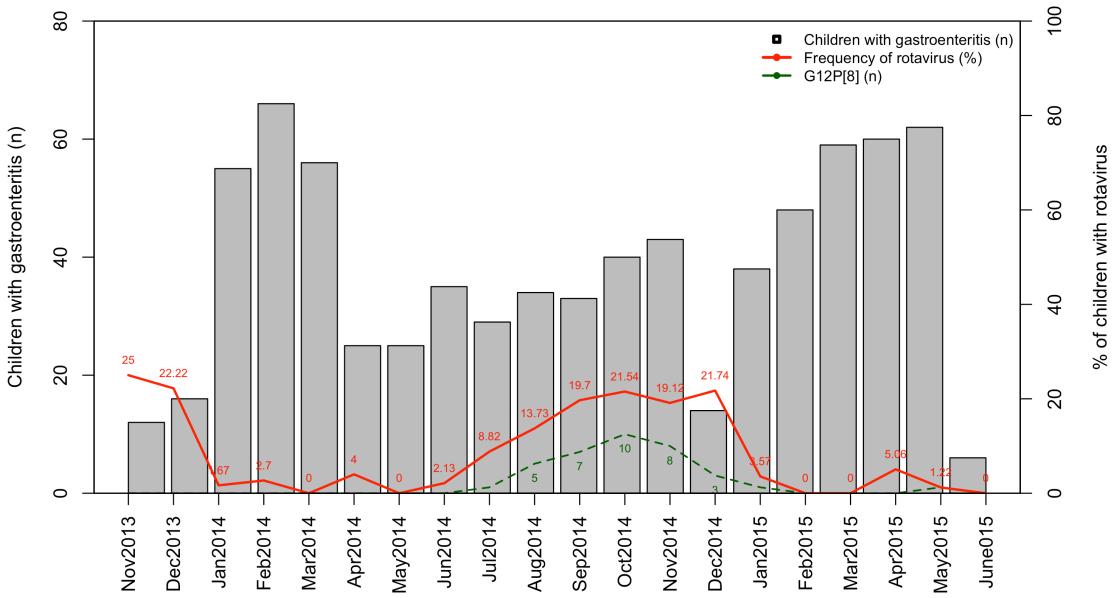


Figure 5. Rotavirus prevalence over study period demonstrating the relationship between rotavirus seasonality (red line) and rotavirus genotype G12P[8] prevalence (green line), Peru, 2013-15



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Yori, Pablo Peñataro, Kellogg Schwab, Robert H Gilman, Sharon Nappier, Daniel Velasquez Portocarrero, Robert E Black, Maribel Paredes Olortegui, et al. 2009. "Norovirus Highly Prevalent Cause of Endemic Acute Diarrhea in Children in the Peruvian Amazon." *The Pediatric Infectious Disease Journal* 28 (9): 844–47. doi:10.1097/INF.0b013e3181a24730.

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Sarah Blythe Ballard, MD, PhD(c), MPH

thenavydoc@gmail.com

Education/ Training
Johns Hopkins School of Public Health—Global Disease Epidemiology PhD, 2011-17
Johns Hopkins School of Public Health—NIH Fogarty Global Health Fellow, 2012-14
Johns Hopkins School of Public Health—Preventive Medicine Residency, 2011-13
Johns Hopkins School of Public Health—Master of Public Health, 2008-10
Naval Aerospace Medicine Institute—Flight Surgery Specialty Training, 2007-08
Naval Medical Center San Diego—Transitional Medical Internship, 2006-07
Emory University School of Medicine—Doctor of Medicine, 2002-06
Agnes Scott College—Bachelor of Arts, Biochemistry & Molecular Biology, 1998-2002

Profile: Navy preventive- and aerospace medicine-boarded physician with humanitarian, injury, and tropical disease research experience submitting this dissertation for the degree of PhD

Employment

Head, Parasitology Department, Naval Medical Research Unit Number Six, 2015-present
- Leads a department of 32 with a $2.5M budget in researching malaria, leishmaniasis, chagas, and intestinal parasites in Peru and other endemic regions around the globe.

Medical Officer in Charge/Flight Surgeon, U.S. Marine Corps Heavy Helicopter Squadron 362, 2010-11
- Supervised 5 flight surgeons and 18 corpsmen in caring for 1,880 deployed Third Marine Aircraft Wing (Forward) Marines as Camp Bastion, Afghanistan, Flight Line Aid Station Senior Medical Officer during Operation Enduring Freedom.
- Managed the medical care of blast-related traumatic brain-injured service members as a Camp Bastion Concussion Restoration Care Center attending physician.
- Coordinated mass casualty medical planning and preparations for the 2010 Afghan elections.
- Served as a Third Marine Aircraft Wing (Forward) disease/non-battle injury surveillance coordinator, health promotions officer, mishap board member, and aviation safety board member.

Medical Department Head/Command Flight Surgeon, U.S. Navy Patrol Squadron Four, 2008-10
- Advised the squadron commander on injury prevention and control strategies, conducted deployment risk assessments, implemented command health policy, supervised troop health education, and planned individual/ mass casualty response and medical evacuation protocols for 500 deployed personnel as Command Flight Surgeon during Operation Iraqi Freedom.
- Developed advanced cardiac life support and combat casualty care curricula for the 10th Iraqi Army at Camp Dhi Qar as Military Liaison to the Dhi Qar Provincial Reconstruction Team.
- Served as mass casualty, medical evacuation, aviation mishap, disaster drill, and medical humanitarian assistance liaison to Combined Joint Special Operations Task Force-Arabian Peninsula, U.S. Air Force Air Expeditionary Group 407, and U.S. Army 4/1 and 2/12 Cavalry units.

Coordinator, U.S. Navy Comprehensive Combat and Complex Casualty Care Center (C5), 2007
- Coordinated care for in- and outpatient complex trauma, combat trauma, and disease/non-battle injury evacuations to Naval Medical Center San Diego from international, national, and local operations.
- Oversaw the prospective casualty management database, tracking demographics, diagnoses, interventions, wound microbiology, and health outcomes for C5 patients.
- Managed primary care for Naval Medical Center San Diego's Wounded Warrior Battalion.

Intern, Centers for Disease Control and Prevention HIV/AIDS and Retrovirology Branch, 2000-02
- Performed HIV vaccine development and evaluation with subtype C viral isolates.
- Investigated the impact of tuberculosis and malaria on HIV-1 replication in cellular compartments in vivo and incorporation of HLA-DR into its envelope.
- Investigated the role of nuclear factor of activated t-cells in the preferential infection and replication of HIV-1 in memory vs. naïve CD4 lymphocytes.

Sarah Blythe Ballard, MD, PhD(c), MPH

thenavydoc@gmail.com

Fellowships

- Fulbright U.S. Student Program, Lima, Peru, 2014-15
- NIH Fogarty Global Health Fellow, UNC-Johns Hopkins-Morehouse-Tulane Consortium, 2012-14
- Paul Farmer Global Health Effectiveness Scholar, Harvard School of Public Health, 2012
- Manfred Wörner Fellow, German Marshall Fund of the United States, 2011
- Asia-Pacific Center for Security Studies Fellow, 2009

Board Certifications and Academic Certificates

- Diplomate, American Board of Preventive Medicine, Aerospace Medicine, 2014
- Diplomate, American Board of Preventive Medicine, General Preventive Medicine, 2013
- ASTMH Certificate of Knowledge in Clinical Tropical Medicine and Travelers' Health, 2012
- Tropical Medicine Diploma Course, Johns Hopkins, 2012
- Conflict Analysis Certificate, U.S. Institute of Peace, 2010
- Negotiation and Conflict Management, US Institute of Peace, 2010
- Interfaith Conflict Resolution Certificate, US Institute of Peace, 2010
- *Strategy and War* Course Certificate, Naval War College, 2010
- Health Finance and Management Certificate, Johns Hopkins, 2010
- Naval Trauma Training Center Advanced Course, Los Angeles County + USC, 2010
- Health Emergencies in Large Populations Certificate, COE-DMHA, 2009
- Combined Humanitarian Assistance Response Training, COE-DMHA, 2008
- Military Tropical Medicine Diploma Course, USUHS, 2007
- Military Medical Humanitarian Assistance Certificate, USUHS, 2007

Faculty Appointments and Teaching Experience

- Adjunct Assistant Professor, Department of Preventive Medicine & Biometrics, Uniformed Services University of the Health Sciences, "**Military Tropical Medicine**," 2012-present, Field Course Director
- Johns Hopkins PH.221.639.81 "**Refugee Health Care,**" 2011-12, Lead Teaching Assistant (TA)
- Johns Hopkins PH.221.613.01 "**Introduction to Humanitarian Emergencies,**" 2011-12, Lead TA
- Johns Hopkins PH.550.609.19 "**Problem Solving in Public Health,**" 2012, 2013, Lead TA
- Johns Hopkins PH.221.661.01 "**Project Development in Developing Countries,**" 2011-12, Lead TA
- Johns Hopkins PH.180.670.01 "**Introduction to Public Health**," 2011-13, Lead TA
- Johns Hopkins PH.180.670.01 "**Health Emergencies in Large Populations**," 2012, Lead TA

Elected/Appointed Positions

- President, American College of Preventive Medicine (ACPM), Resident Physician Section, 2013-14
- Vice President for Policy and Education, ACPM Resident Physician Section, 2012-13
- Board of Directors, Uniformed Services Academy of Preventive Medicine, 2012-15
- Board of Governors, *Ex-officio*, American Journal of Preventive Medicine, 2012-13
- Young Professional Board Member, American Red Cross of Central Maryland, 2011-13
- Health Committee Member, American Red Cross, Hawaii State Chapter, 2008-11
- Pandemic Preparedness Officer, Marine Corps Base Hawaii, 2009-10
- Education and Training; Aviation Safety Committee Aerospace Medical Association, 2008-09; 2012-14

Humanitarian Assistance/Disaster Relief Field Work

- Medical Coordinator, Ebola Rapid Response Initiative, International Medical Corps, 2014-15
- Human Rights Clinic Physician, HealthRight International, 2011-13
- Disaster Action Team, Young Professionals Board, American Red Cross of Central Maryland, 2011-13
- Emergency Doctor, Disaster Action Team, certified in Disaster Assessment, Health Services, Mass Care, Shelter, and Logistics Volunteer, American Red Cross, Hawaii State Chapter, 2008-11

- Navy Medical Liaison, Dhi Qar Provincial Reconstruction Team, Iraq, 2008-09
- Supervising Flight Surgeon, Combined Joint Task Force-Horn of Africa, for three Medical Civic Action Immunization/Primary Care Project Missions along the Djiboutian-Somali-Ethiopian borders, 2008
- Military Medical Relief Worker for 8.0 earthquake in Pisco, Peru, 2007

Research & Service Awards

- Ashton Graybiel Award for impactful aviation research (2015)—Society of U.S. Naval Flight Surgeons
- TIME Magazine Person of the Year (2014)—Ebola Fighters
- Elsevier Clinical Research Award (2013) – American Society of Tropical Medicine and Hygiene
- American Society of Tropical Medicine and Hygiene Travel Award (2013)
- Young Investigator Award Finalist (2013) – Aerospace Medical Association, top 7% of 206 abstracts
- Aerospace Medicine Students and Residents Organization (2013) – AsMA Conference Travel Award
- John Paul Stapp Prize in aviation research (2012) –Johns Hopkins Center for Injury Research and Policy

Selected Military Decorations/Awards

- Richard E. Luehrs Operational Flight Surgeon of the Year, Navy-Marine Corps (2010)
- Chief of Naval Operations Safety Award (2010)
- Overseas Service Ribbon (2015)
- Navy "Blue M" Outstanding Fleet Medical Readiness Awards (2008 and 2009)
- Combat Patrol and Reconnaissance Wing TWO Flight Surgeon of the Year (2008 and 2009)
- US Navy Surgeon General's "Blue H" Health Promotions Awards (2008 and 2009)
- U.S. Naval Flight Surgeon Qualification (2008)
- Fleet Marine Force Qualified Officer (2011)
- Individual Strike/Flight Air Medals (2009 and 2011)
- Afghan Campaign Medal (2010)
- North Atlantic Treaty Organization Medal (2010)
- Outstanding Volunteer Service Medal (2010)
- Navy and Marine Corps Achievement Medals (2008 and 2010)
- Iraq Campaign Medals – Iraqi Surge (2008) and Iraqi Sovereignty (2009)
- Sea Service Medals (2009 and 2010)
- Advanced Rotary Wing (Bell TH-57 Jet Ranger) Aviation, US Navy (2008)
- Primary Fixed Wing (Beechcraft T-34 Turbo Mentor) Aviation, US Navy (2008)
- Aviation Preflight Indoctrination, US Navy (2007)
- Humanitarian Service Medal (2007), Letter of Commendation, US Ambassador to Peru (2007)

Military Extracurricular Leadership Activities

- Mentor for "Academy Women," a non-profit global leadership organization for current and former military officers, cadets, midshipmen, candidates, and allies to support women leadership (2008-present)
- Interviewer, Health Professions Scholarship Program (2011-present)
- Field Clinical Preceptor, Military Tropical Medicine Civic Action Project, Peru (2012-present)
- Navy Delegate, Hawaii Governor's International Leadership Conference (2008, 2009)

Academic Honors

- Delta Omega, Alpha Chapter, Johns Hopkins (2010)
- Phi Beta Kappa, Agnes Scott College (2002)
- Agnes Scott College Outstanding Young Alumnus (2009)
- Agnes Scott College Mortar Board (2002)

Ongoing Research Support

P0376-14-N6 Ballard (PI) 10/01/14-10/01/16
Department of Defense Global Emerging Infections Surveillance System Program $182,000
Role of loggers in malaria re-introduction/ re-emergence in low endemic areas in the Peruvian Amazon

20160390130 Ballard (PI) 10/01/15-10/01/16
Department of Defense Global Emerging Infections Surveillance System Program $415,000
Malaria surveillance sustainment

Completed Research Support

P0067-13-N6 Ballard (PI) 10/01/13-10/01/15
Department of Defense Global Emerging Infections Surveillance System Program $199,800
In vivo efficacy of artemisinin combination therapy in Peru to determine parasite clearance rates in uncomplicated *P. falciparum* malaria

Procter & Gamble Fellowship Ballard (PI) 08/01/14-05/31/15
Johns Hopkins Bloomberg School of Public Health Endowed Research Award
$17,780.00
Scholarship awarded to students committed to advancing the health and well-being of women and children through the provision of clean water and improved nutrition.

U.S. Fulbright Research Award – Peru Ballard (PI) 08/01/14-04/30/15
United States Department of State $9,000.00
The goal of this award is to increase mutual understanding between people of the United States and the people of Peru through intellectual exchange of technology and epidemiological research skills.

R. Bradley Sack Family Scholarship Ballard (PI) 06/01/14-05/31/15
Johns Hopkins Bloomberg School of Public Health Endowed Research Award
$4,350.00
This fund supports outstanding doctoral students studying infectious diseases in the developing world.

Early Career Award Ballard (PI) 02/02/14-01/31/15
Thrasher Research Fund
$25,000.00
The goal of this award is to encourage the development of young investigators in pediatric medical research. It supports the evaluation of medically-attended norovirus-associated diarrhea in Peru.

Centennial Travel Award Ballard (PI) 09/15/13-09/14/14
American Society of Tropical Medicine and Hygiene $25,000.00
The goal of this award is to provide short-term research experience in the tropics. It funds technical personnel and equipment for the norovirus hospital study.

Graduate Research Award Program Ballard (PI) 08/01/13-07/31/14

National Academies of Science, Transportation Research Board $10,000.00
The goal of this fund is to encourage applied research on airport and related aviation system issues and to foster the next generation of aviation community leaders.

David and Elinor Bodian Foundation Ballard (PI) 09/01/13-05/16/14
Johns Hopkins Bloomberg School of Public Health Endowed Research Award
$3,500.00
The goal of this award is to support the research of a student whose work is at a critical juncture.

Clements-Mann Fund in Vaccine Sciences
09/01/13-05/16/14
Johns Hopkins Bloomberg School of Public Health Endowed Research Award
$4,500.00
The goal of this fund is to support research for outstanding students and residents working in vaccine sciences.

Henry K. and Lola Beye Award 09/01/12-05/16/13
Johns Hopkins Bloomberg School of Public Health Endowed Research Award
$3,500.00
The goal of this fund is to support research for outstanding students and residents in global health.

R25 TW009340 Van Der Horst (PI) 07/15/12-05/14/14
Fogarty International Center – UJMT Consortium
$28,000.00
The goal of this award is provide supportive mentorship and research opportunities for early stage investigators. It provides a stipend and $14,000 per year for the norovirus hospital study.
Role: Fogarty Global Health Fellow (receiving support)

847705 8200 25GB B0016 Ballard (PI) 07/01/12-09/30/13
Department of Defense Global Emerging Infections Surveillance Program $20,000.00
The goal of this study was to evaluate the epidemiology of norovirus infection in a Peruvian military recruit cohort in the Peruvian Amazon.

Global Health Established Field Placement Award
08/01/12-10/31/12
Johns Hopkins Center for Global Health $3,500.00
The goal of this award is to provide residents and students with means to attain international cross-cultural field experience. It funded living expenses for the Peruvian military norovirus study.

Simpson Tropical Medicine Student Award 08/01/12-10/31/12
Tropical Medicine Dinner Club of Baltimore
$500.00
The goal of this award is to provide a student or resident with funding to pay for travel expenses related to the conduct of tropical medicine research in a developing setting.

Sarah Blythe Ballard, MD, PhD(c), MPH

thenavydoc@gmail.com

Bibliography

Journals

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Ballard SB, Osorio VB. U.S. civil air show crashes, 1993-2013: burden, fatal risk factors, and FIA Score evaluation. *Transportation Research Record* 2015;2471:1-9.

Ballard, SB. The U.S. commercial air tour industry: a review of aviation safety concerns. *Aviat Space Environ Med* 2014;85(2):160-6. PMID: 24597160

Ballard SB, Beaty LP, Baker SP. Hot-air balloon tours: crash epidemiology in the US, 2000-2011. *Aviat Space Environ Med* 2013;84(11):1172-7. PMID: 24279231

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Pisell,TL, Hoffman IF, Jere CS, **Ballard SB**, Molyneux ME, Butera ST, et al. Immune activation and induction of HIV-1 replication within CD14 macrophages during acute Plasmodium falciparum malaria coinfection. *AIDS*. 26 July 2002; 16(11): 1503-9. PMID: 12131188

Databases

Ballard, SB. Microcoil embolization of a lower GI bleed. *MedPix Database Engine.* 2006. Available from: URL: https://rad.usuhs.mil/medpix/index.html?mode=factoid_images&recnum=6769.

Invited Presentations

Smith ES, Durand S, Tapia SL, Cabezas C, Pachas PE, Sihuincha M, Edgel KA, Baldeviano GC, Lescano AG, **Ballard SB**. *Plasmodium falciparum* parasite clearance in the Peruvian Amazon as part of a DoD Harmonized Clinical Trial. A65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016. ***1st Place Elsevier Clinical Research Award***

Ballard SB. Targeting populations for Norovirus immunizations. Curso Internacional de Enfermedades Tropicales e Infecciosas: Hacia el Control de Enfermedades Transmisibles. August 2015.

Ballard, SB. Targeting populations for Norovirus vaccination. Innovations in Global Health Seminars, Kuskaya Programa de Formación Interdisciplinaria en Innovaciones para la Salud Global, Universidad Peruana Cayetano Heredia, May 2015.

Colquechagua Aliaga FD, Rapoport LB, Mirelman AJ, Tejada MG, Rafael Cordova BM, Figueroa Quintanilla DA, Gilman RH, **Ballard SB.** Inseguridad alimentaria y los costos del tratamiento medico de la gastroenteritis en niños Peruanos menores de cinco años de edad en un period post-vacunal contra rotavirus [abstract]. American Society of Tropical Medicine and Hygiene – Latin America, February 2013.

Rapoport LB, Colquechagua Aliaga FD, Mirelman AJ, Tejada MG, Rafael Cordova BM, Figueroa Quintanilla DA, Gilman RH, **Ballard SB**. Indirect costs of medically-attended gastroenteritis in children younger than five years of age in a "post-rotavirus" setting [abstract]. 63rd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2014.

Ballard SB. Noroviruses in Peru: an update. Curso Internacional de Enfermedades Tropicales e Infecciosas: Hacia el Control de Enfermedades Transmisibles. August 2014.

Ballard SB. The U.S. commercial air tour industry: what's wrong, and how do we fix it? 85th Annual Scientific Meeting of the Aerospace Medical Association [abstract]. Aviat Space Environ Med, May 2014.

Ballard SB, Luna CG, Apaza S, Espetia S, Saito M, Silva ME, Reaves EJ, Rocha C, Vilela KJ, Tilley DH, Blazes DL, Gilman RH, Bausch RG. Genetic characterization of noroviruses among Peruvian army recruits in the amazon basin. 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2013. ***2nd Place Elsevier Clinical Research Award***

Ballard SB. Rotavirus and Norovirus: Science, public health implications, and common pathways to control. American Society of Tropical Medicine & Hygiene Diploma Course, Johns Hopkins Summer Institute in Tropical Medicine, July 2013.

Ballard SB. Norovirus-associated diarrhea: Mission-aborting potential and security threats. UPMC Center for Health Security, May 2013.

Baker SB, **Ballard SB**, Beaty LP. Hot-air balloon tours: Crash epidemiology in the US, 2000-2011. 84th Annual Scientific Meeting of the Aerospace Medical Association [abstract]. Aviat Space Environ Med 2013:84(4):295.

Ballard SB. Norovirus epidemiology and control: The changing pathogen landscape in Peru. Simpson Student Lecturer at the Tropical Medicine Dinner Club of Baltimore, February 2013.

Ballard SB. Norovirus epidemiology, genetics, immunology, and control: A review. Johns Hopkins MPH Capstone Presentation, Johns Hopkins Bloomberg School of Public Health, May 2010.

Ballard SB. Norovirus epidemiology and military importance. Johns Hopkins Human Viral Infections Course, Johns Hopkins Bloomberg School of Public Health, February 2009.

Ballard SB. Cross-border healthcare to reduce maternal mortality in Badakshan, Afghanistan. Johns Hopkins Institute in Health Policy and Management, Universitat Pompeu Fabra, November 2009.

Ballard SB. HIV-1 origin determination by immunomagnetic capture. Agnes Scott College Spring Annual Research Conference, April 2001.

Posters

Ballard SB, Jennings MC, Steinberg HE, Tilley DH, Meza R, Villanueva M, Maldonado Costa F, Lopez M, Luna CG, Silva ME, Maves RC, Lescano AG, Cabada MM, Simons MP. Antimicrobial resistance patterns among intermediate- to long-term travelers to Cusco, Peru, with diarrhea. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Pan W, Zaitchik B, Pizzitutti F, Feingold B, Mena C, Recalde C, Salmon-Mulanocich G, Vidal E, Smith E, **Ballard SB**. Developing an early warning system for malaria in the Amazon: Progress in Peru. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Oyola Lozada G, **Ballard SB**, Ochoa Porras M, Sanchez Garcia G, Colquechagua Aliaga FD, Zamuido Zeaa R, Bern C, Saito M, Figueroa Quintanilla D, Gilman RH, Mayta H. Rotavirus among medically-attended children younger than fiver years of age with and without diarrhea in Lima, Peru, following universal rotavirus vaccine implementation. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Salas CJ, Barazorda KA, Lucas CM, Tapia LL, Valdivia HO, **Ballard SB**, Gerbasi RV. Establishment of the Ring-stage Survival Assay (RSA) approach to monitoring artemisinin antimalarial drug resistance in the Peruvian Amazon Basin. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Barazorda KA, Salas CJ, Durand S, Lucas CM, **Ballard SB**, Gerbasi RV. Absence of *Plasmodium falciparum* K13 propeller SNPs 15 years after incorporating ACTs into treatment guidelines in the Peruvian Amazon. 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Sanchez GJ, Prado A, Gilman RH, Neira KE, Oyola MG, Ochoa MR, Vittet MD, Fernandez JM, Villegas MC, Coronado NS, Sancho C, Colquechagua FD, **Ballard SB**, Saito M, Mayta H. Multiplex and high resolution melting point PCR for sapovirus genotyping. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Pinedo Cancino V, Baldeviano GC, Arista KM, Smith ES, Ventocillo JA, Pinedo S, Franco A, Arana A, Gerbasi RV, **Ballard SB**, Lescano AG, Ruiz-Mesia L. Assessing malaria transmission intensity in a low endemic area of the Peruvian Amazon using parasitological and serological surveys. 65th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2016.

Jennings MC, Tilley DH, **Ballard SB**, Villanueva M, Maldonado Costa F, Lopez M, Luna CG, Meza R, Silva ME, Simons MP, Maves RC, Cabada MM. Case-case analysis of enteric diseases using travelers' diarrhea surveillance data: applications in Cusco, Peru. Annual Meeting of the American College of Preventive Medicine, February 2016.

Ballard SB, Sanchez G, Mayta M, Colquechagua FD, Jahuira MH, Bern C, Oyola MG, Cabrera L, Tilley DH, Saito M, Figueroa D, Gilman RH. Norovirus and sapovirus in medically-attended children presenting with and without gastroenteritis in an LMIC setting following rotavirus vaccine implementation. 64th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2015.

Ballard SB, Cowie SR, Mullins K, Waggoner PD, Wilkie JJ. Short-range air transportation of Ebola suspect cases: an evidence-based protocol. 3rd International Congress on Infectious Diseases—Targeting Ebola 2015, Institut Pasteur, May 2015.

Prudhomme MB, **Ballard SB**. Protocol for diagnosis and treatment of undifferentiated febrile illness by a forward-deployed military medical provider. 100th Annual Meeting of the American College of Occupational and Environmental Medicine, May 2015.

Alsentzer E, Vera DM, Neyra J, Loayza L, Hora RA, Osorio B, Quispe J, **Ballard SB**, Blazes DL. Monitoring acute diarrhea via an electronic surveillance system in the Peruvian Navy. International Society for Disease Surveillance 2014 Conference Abstracts. *Online Journal of Public Health Informatics* 2015;7:1.

Pollett S, **Ballard SB**, Eden JS, Luna G, Espetia S, Silva M, Reaves E, Tilley DH, Simons M, Guzman RC, Holmes EC, Gilman RH, Bausch DG. The molecular epidemiology of norovirus in military recruits in Iquitos, Peru: evidence of a novel genotype. 63rd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2014.

Ballard SB, Reaves EJ, Luna CG, Silva ME, Gilman RH, Bausch DG. A case-control study of norovirus-induced acute diarrhea among Army recruits in Peru. 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2013.

Ballard SB, Beaty LP, Baker SP. US commercial air tour crashes, 2000-2011: Public health burden, fatal risk factors, and FIA score validation. 84th Annual Scientific Meeting of the Aerospace Medicine Association [abstract]. *Aviat Space Environ Med* 2013:84(4):359.

Ballard SB, Reaves EJ, Luna CG, Gilman RH, Bausch DG. Norovirus epidemiology in a 6-year Peruvian Amazon military cohort. Johns Hopkins Center for Global Health. Center for Global Health poster competition, April 2013.

Ballard SB, Reaves EJ, Luna CG, Silva ME, Bausch DG, Gilman RH. Endemic norovirus is associated with adult diarrhea in a developing setting. NIH Fogarty Global Health Fellows Conference, July 2013.

Ballard SB, Beaty LP, Baker SP. The epidemiology of occupational injuries and deaths among US commercial air tour pilots, 2000-2011. Annual Meeting of the American College of Preventive Medicine, February 2013.

Ballard SB. Isolation of CHO cells producing MIP-3α and MIP-3α IgG. Emory Medical School Research Conference, May 2003.